February 2023

QIAseq® xHYB Viral and Bacterial Library Kit Handbook

Sample to Insight® solution for high-throughput targeted next-generation sequencing of microbial samples

Sample to Insight

Contents

Kit Contents

QIAseq xHYB Respiratory Panel QIAseq xHYB Viral STI Panel QIAseq xHYB AMR Panel QIAseq xHYB Adventitious Agent Panel	(24) 333322 333332 333342 333352	(96) 333325 333335 333345 333355
Box 1 of 3 (Number of samples)	(24)	(96)
QIAseq xHYB Panel, probes	30 µL x 1	116 µL x 1
Post Hybrid-Capture PCR Mix 2x	660 µL x 1	660 µL x 3
Primer Mix Illumina® Libr. Amp	20 µL x 2	180 µL x 1
Hyb Mix	150 µL x 1	580 µL x 1
One-4-All Blocking Solution	15 µL x 2	60 µL x 2
One-4-All Blocking Oligos	24 µL x 2	96 µL x 2
Vapor-Lock	500 µL x 1	500 µL x 2
Hyb Elute Buffer	400 µL x 1	1590 µL x 1
Enhanced Blocking Buffer	18 µL x 1	85 µL x 1
Box 2 of 3 (Number of samples)		
Microbial RP	30 µL x 1	116 µL x 1
BC3 buffer	48 µL x 1	384 µL x 1
RI RNase Inhibitor	12 µL x 2	144 µL x 1
EZ Reverse Transcriptase	36 µL x 1	150 µL x 1
XC buffer, 10x	24 µL x 2	192 µL x 1
RH RNase	12 µL x 2	96 µL x 1
dNTP	12 µL x 2	96 µL x 1
BX enzyme	12 µL x 2	96 µL x 1
UPH Ligation Buffer	288 µL x 2	1152 µL x 2
Lib Amp Blocker	30 µL x 1	116 µL x 1

QIAseq xHYB Respiratory Panel QIAseq xHYB Viral STI Panel QIAseq xHYB AMR Panel QIAseq xHYB Adventitious Agent Panel	(24) 333322 333332 333342 333352	(96) 333325 333335 333345 333355
Box 3 of 3 (Number of samples)	(24)	(96)
Binding Buffer	6.25 mL x 1	25 mL x 1
Wash Buffer 1	3.25 mL x1	13 mL x 1
Wash Buffer 2	5.0 mL x 1	19 mL x 1
Streptavidin Binding Beads	1200 µL x 1	1200 µL x 2
QIAseq Beads	10 mL x 1	38.4 mL x 1
Nuclease-Free Water	10 mL x 1	50 mL x 1

QIAseq xHYB MPXV Spike-In Panel Catalog. no.	(24) 333392	(96) 333395
Box 1 (Number of samples)	(24)	(96)
QIAseq xHYB Panel, probes	30 µL x 1	116 µL x 1

QIAseq xHYB MPXV Panel Catalog no.	(96) 333397
Box 1 of 2 (Number of samples)	(96)
QIAseq xHYB Panel, probes	116 µL x 1
Post Hybrid-Capture PCR Mix 2x	660 µL x 3
Primer Mix Illumina Libr. Amp	180 µL x 1
Hyb Mix	580 µL x 1
One-4-All Blocking Solution	60 µL x 2
One-4-All Blocking Oligos	96 µL x 2
Vapor-Lock	500 µL x 2
Hyb Elute Buffer	1590 µL x 1
Enhanced Blocking Buffer	85 µL x 1
UPH Ligation Buffer	1152 µL x 2
Lib Amp Blocker	116 µL x 1
Box 2 of 2 (Number of samples)	(96)
Binding Buffer	25 mL x 1
Wash Buffer 1	13 mL x 1
Wash Buffer 2	19 mL x 1
Streptavidin Binding Beads	1200 µL x 2
QIAseq Beads	38.4 mL x 1
Nuclease-Free Water	50 mL x 1

Shipping and Storage

The QIAseq xHYB Viral and Bacterial Library Kits are shipped in three boxes. Boxes 1 and 2 are shipped on dry ice or blue ice, and Box 3 is shipped on blue ice. Upon receipt, all the components in Boxes 1 and 2 should be stored immediately at –30 to –15°C in a constant-temperature freezer. All the components in Box 3, the beads, and wash buffers should be stored immediately at 2–8°C. QIAseq xHYB MPXV Spike-in Panel or stand-alone Panel Box 1 is shipped on dry ice or blue ice, and Box 2 is shipped on blue ice. Upon receipt, all the components in Box 1 should be stored immediately at –30 to –15°C in a constant-temperature freezer. All components in Box 2, the beads, and wash buffers should be stored immediately at 2–8°C.

Intended Use

The QIAseq xHYB Viral and Bacterial Library Kits are intended for molecular biology applications. These products are not intended for the diagnosis, prevention, or treatment of a disease.

All due care and attention should be exercised in the handling of the products. We recommend all users of QIAGEN® products to adhere to the NIH guidelines that have been developed for recombinant DNA experiments, or to other applicable guidelines.

Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate safety data sheets (SDSs). These are available online in convenient and compact PDF format at **www.qiagen.com/safety**, where you can find, view, and print the SDS for each QIAGEN kit and kit component.

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of the QIAseq xHYB Library Kit is tested against predetermined specifications to ensure consistent product quality.

Introduction

Microbial genomes and genes are highly diverse, and this is especially true of viral genomes. Viral genomes exhibit high diversity in morphology, genome size, and genomic organization, as they can be composed of DNA or RNA, and in the case of retroviruses, both. In addition, some viruses can exist as an episome or integrate into the host genome. Genome sizes for human viral pathogens can vary from a few thousand to several hundred thousand bases. Also, they can be organized in a single DNA or RNA molecule or can be segmented into several molecules as in Influenza, which contains 8 segments.

In addition to viral genomes, bacterial antimicrobial resistance genes are also highly diverse, reflecting the large number of antibiotic drug classes and mechanisms used for resistance. While deep sequencing is capable of detecting antimicrobial signatures, it lacks the sensitivity of targeted sequencing approaches.

There are a number of challenges to sequencing microbial genes or genomes. The targeted viral genome or bacterial gene may be present at low copy numbers especially relative to host and commensal genomes. Additionally, viral genomes and bacterial gene sizes are more than 1 order smaller than the host genome and/or the metagenome. This can lead to a very small proportion of reads allocated to the target, which reduces sensitivity in detection. Also, viral genomes have a relatively high mutation rate, which can limit the effectiveness of amplicon-based enrichment methods. This is due to SNPs from non-annotated strains or newly emerging strains that may prevent primer extension and amplicon generation. Amplicon-based targeted panels for either viral genomes or antimicrobial resistance genes are rarely comprehensive due to the high number of primer amplicon designs required. Finally, if a viral genome is integrated into the host-genome, which can occur in latent infection, this is refractory to standard 2 primer amplicon-based enrichment.

To address the challenges of sequencing microbial genes and genomes, hybrid capture panels have been developed to produce high quality microbial libraries. The hybrid capture panels are used with libraries that are generated after converting RNA to double-stranded cDNA, and then amplified and indexed using the QIAseq FX DNA Library Kit with Unique Dual Indexes (UDIs). Hybrid capture enriches for the target sequence while allowing tolerance for mismatches that may occur in non-annotated or emerging strains. In addition, hybrid capture allows the efficient targeting of a panel of viral genomes or bacterial genes since the number of probes can be scaled up in a cost-effective manner.

Principle and procedure

The QIAseq xHYB panels utilizes a workflow that consists of library construction followed by hybrid capture for targeted enrichment of viral genomes or bacterial antimicrobial resistance (AMR).

Figure 1A: Schematic of double-stranded cDNA synthesis and QIAseq FX DNA Library Kit Workflow

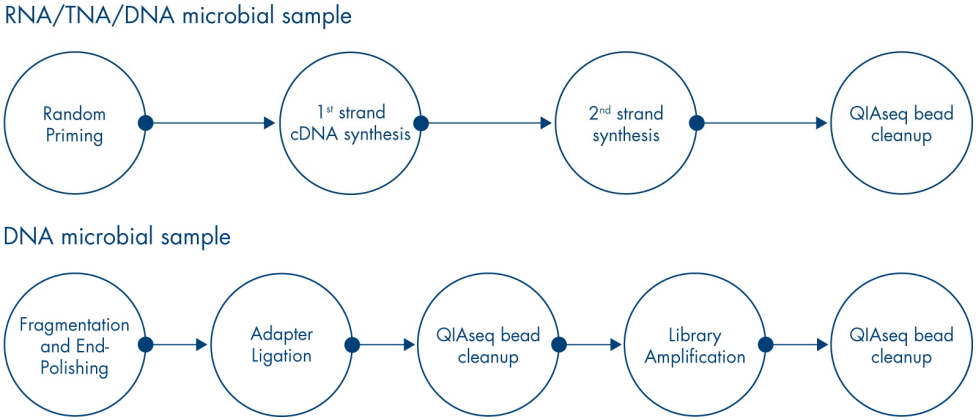

Figure 1B: Schematic of hybridization targeted enrichment

Figure 1. Scheme of optimized QIAseq xHYB library construction and target enrichment. (A) The QIAseq xHYB workflow illustrating the conversion of microbial RNA/TNA/DNA samples to double-stranded cDNA. After QIAseq bead cleanup, the double-stranded cDNA product is the template for the QIAseq FX DNA Library Kit. Alternatively, if starting with microbial DNA samples, the workflow can start with Fragmentation/end-polishing step. Excess adapters are removed by QIAseq bead cleanup. **(B)** The purified libraries are then pooled and concentrated by drying down. The dried-down libraries are hybridized with biotinylated probes overnight. After washing, library amplification and a QIAseq bead cleanup result in sequencing-ready libraries.

Converting RNA into double-stranded cDNA

The QIAseq xHYB workflow begins with converting total RNA in the sample into cDNA. The reaction is flexible with regards to input RNA into the reverse transcription reaction as up to 5 µL of total RNA can be added. The reverse transcription product is then converted into double-stranded cDNA, which is then used as the input for QIAseq FX DNA Library construction, after a QIAseq bead cleanup.

QIAseq FX DNA Library construction

The purified cDNA product is converted to Illumina-compatible NGS libraries using the QIAseq FX DNA Library Kit. The QIAseq FX kit will also generate libraries from genomic DNA if the sample contains it (either as total nucleic acid or isolated genomic DNA). Purified double-stranded DNA products are enzymatically sheared, the fragmented DNA is end-repaired, and an "A" is added to the 3'-end. The product is ready for adapter ligation where Illumina-platform–specific adapters are ligated to both ends of the DNA fragments. The adapters contain the necessary sequences to allow libraries to bind to the flow-cell for sequencing. Following adapter ligation, the libraries are purified using QIAseq Beads, which remove any free adapters. The libraries are then amplified to generate sufficient yields that will go into the hybrid capture reaction.

Hybrid capture targeted enrichment

The purified libraries are pooled with equal mass from each library. In addition, Enhanced Blocking Buffer is added to further prevent non-specific hybridization. The pooled libraries are then dried-down using a SpeedVac system, or alternatively, via QIAseq Beads. The dried-down pooled libraries are then resuspended and denatured. Meanwhile, in a separate tube, the QIAseq xHYB panel is mixed with Hybridization Mix and denatured. After both the pooled libraries and QIAseq xHYB panel cool, the xHYB panel is added to the pooled libraries and this is placed overnight in a thermal cycler where the probes will hybridize to the targets. After

overnight incubation, the biotinylated probes, along with any captured products, are bound to streptavidin-coated beads. The bound probes and streptavidin beads are washed to remove any unbound library fragments and the streptavidin-bound library is resuspended with Hyb Elute Buffer. A post-hybrid capture amplification is performed to convert single-stranded DNA into Illumina sequencer-compatible double-stranded libraries. A final QIAseq Beads purification is performed, and final libraries are ready for sequencing.

Next-generation sequencing

After the QIAseq xHYB Libraries have been quantified with the QIAseq Library Quant Array or Assay Kit, the libraries are compatible with Illumina NGS platforms including iSeq® 100, MiniSeq®, MiSeq®, NextSeq® 500/550, HiSeq® 1000, HiSeq 1500, HiSeq 2000, HiSeq 2500, HiSeq 3000/4000, and NovaSeq® 6000. When using Illumina NGS systems, 149 bp paired-end reads are required.

Important: As a starting point, we recommend allocating 1M clusters per sample. Table 29 describes the number of samples that can be multiplexed.

Data analysis

A simplified downstream data analysis is available through QIAGEN's GeneGlobe® Data Analysis portal. This provides identification and quantification of either viral species or antimicrobial resistance genes detected in all any samples. A deeper analysis is available with CLC Genomics Workbench. In addition to identification, variants, phylogenetic trees, viral integration sites, etc. are generated for further analysis.

Equipment and Reagents to Be Supplied by User

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate safety data sheets (SDSs), available from the product supplier.

Required products

- QIAseq FX DNA Library UDI Kit (QIAGEN, cat. no. 180477, 180479, 180480, 180481, or 180482)
- **Preferred Library Quantification Method**: qPCR instrument and QIAseq Library Quant System: GeneRead Library Quant Array (QIAGEN, cat. no. 333304) or QIAseq Library Quant Assay Kit (QIAGEN, cat. no. 333314)

Consumables and reagents

- Nuclease-free pipette tips and tubes
- 1.5 mL LoBind® tubes (Eppendorf, cat. no. 022431021)
- PCR tubes (0.2 mL individual tubes or tubes strips) (VWR, cat. no. 20170-012 or 93001-118)
- Ice
- 100 % ethanol (ACS grade)
- Nuclease-Free Water

Laboratory equipment

- Single-channel pipettor
- Multichannel pipettor
- Microcentrifuge
- Thermal cycler

- Vortexer
- MagneSphere® Technology Magnetic Separation Stand (Promega, cat. no. Z5342) or DynaMag™-96 Side Magnet (Thermo Fisher Scientific, cat. no. 12331D)
- Vacuum Concentrator (Thermo Fisher Scientific, SpeedVac Vacuum Concentrator System)
- Agilent® 2100 Bioanalyzer®: High Sensitivity DNA Kit (Agilent, cat. no. 5067-4626) or Agilent 4200 TapeStation®: High Sensitivity D1000 ScreenTape (Agilent, cat. no. 5067-5584)
- Heat block that holds 1.5 mL tubes
- Rotator that holds 1.5 mL tubes

Table 1. Optional positive nucleic acid controls from ATCC

Target	Catalog number	Panel
Monkeypox virus	VR-3371D	MPXV
Human coronavirus 229E	VR-740DQ	Adventitious Agent, Respiratory
HPV-16	45113D	Adventitious Agent, STI
Severe acute respiratory syndrome-related coronavirus 2	VR-1986D	Adventitious Agent, Respiratory
HBV (integrated into human genome)	CRL-2235D	Adventitious Agent, STI
Klebsiella pneumoniae	BAA-1706D-5	AMR
Staphylococcus aureus	700699D-5	AMR

Important Notes

General precautions

- Use good laboratory practices to minimize cross-contamination of nucleic acid products.
- Always use PCR tubes, microcentrifuge tubes, and pipette tips that are certified sterile, DNase-free, and RNase-free.
- Before starting, wipe down work area and pipettes with an RNase and DNA cleaning product such as RNase Away® (Molecular Bio-Products, Inc., San Diego, CA) or LookOut® DNA Erase (Sigma-Aldrich).
- For consistent library construction and hybridization reactions, ensure that the thermal cycler used in this protocol is in good working order and has been calibrated according to the manufacturer's specifications.
- Please read the entire protocol before beginning. Take note of the required product, notes, recommendations, and stopping points.
- Recommended library quantification method:
  QIAGEN's QIAseq Library Quant Assay Kit (QIAGEN, cat. no. 333314) or GeneRead Library Quant Array (QIAGEN, cat. no. 333304), which contains laboratory-verified forward and reverse primers together with a DNA standard, is highly recommended for accurate quantification of the prepared QIAseq xHYB libraries.
- Indexing recommendations:
  Sample multiplexing is one of the most important NGS tools for increasing throughput and reducing costs. It works by combining multiple samples to be processed together in a single sequencing run; as a consequence, sequencing reads need to be demultiplexed by reassigning each single read to its original source library. This is facilitated by the integration of index sequences into the individual adapter molecules. Use different sample indexes for all samples. This is achieved using the QIAseq UDI Y-Adapters to generate QIAseq xHYB libraries. The QIAseq FX DNA Library adapters are described in Appendix C.

Protocol: Converting RNA to Double-Stranded cDNA

Important points before starting

- Use high quality RNA/TNA/DNA samples.
- A range of 10 to 100 ng of RNA/TNA/DNA is recommended as input into the random priming reaction. If viral nucleic acids were extracted with carrier RNA, simply use a fixed volume (1–5 µL).
- Ensure reactions are thoroughly mixed, as well as prepared and incubated at the recommended temperatures.
- 80% ethanol should be freshly prepared using Nuclease-Free Water and mixed thoroughly by vortexing.
- Before cleanup, ensure that the QIAseq Beads have been equilibrated to room temperature for 30 minutes.
- **Important**: QIAseq Beads need to be homogenous. Thoroughly resuspend the beads immediately before use and process the beads quickly. If a delay in the protocol occurs, simply vortex the beads again.
- Pre-program thermal cyclers. For increased speed and convenience, all incubation steps of the protocol can be preprogrammed and saved in advanced. Additionally, the program can be started and paused while setting-up the reaction.

Double-stranded cDNA synthesis

1. Thaw reagents and samples on ice. Once reagents are thawed, mix buffers thoroughly by quick vortexing to avoid any localized concentrations. Briefly spin down vortexed reagents.

2. On ice, add up to 5 µL of RNA, TNA, or DNA sample per well. Add each of the remaining components in Table 2 for each sample. Mix well by pipetting up and down 10 times and then spin down briefly.

Table 2. Preparation of random priming reaction

Component	Per sample (µL)
Microbial RNA, TNA, or DNA sample	x
Microbial RP	1
BC3 buffer	2
Nuclease-Free Water	5–x
Total volume	**8**

3. Incubate the reactions in a thermal cycler as described in Table 3.

Table 3. Random priming reaction

Temperature	Time (min)
65°C	5
Ice	>2

4. Remove the tubes/plate from the thermal cycler and briefly centrifuge.
5. On ice, add the components in Table 4 to each sample. Mix well by pipetting up and down 10 times and spin down briefly.

Table 4. First strand synthesis

Component	Per sample (µL)
Random Priming reaction	8
RI RNase Inhibitor	1
EZ Reverse Transcriptase	1
Total volume	**10**

6. Incubate the reactions in a thermal cycler as described in Table 5.

Table 5. First strand synthesis reaction

Temperature (°C)	Time
25	10 min
42	30 min
70	15 min
4	Hold

7. Remove the tubes/plate from the thermal cycler and briefly centrifuge.
8. On ice, add the components in Table 6 to each sample. Mix well by pipetting up and down 10 times and spin down briefly.

Table 6. Second strand synthesis

Component	Per sample (µL)
Reverse Transcription reaction	10
Nuclease-Free Water	5
XC buffer	2
RH RNase	1
dNTP	1
BX enzyme	1
Total volume	**20**

9. Incubate the reactions in a thermal cycler as described in Table 7.

Table 7. Second strand synthesis reaction

Temperature (°C)	Time
37	7 min
65	10 min
80	10 min
4	Hold

Cleanup after second strand synthesis with QIAseq Beads

1. Add 30 µL H_2O to each second strand reaction.
2. Add 65 µL (1.3x) QIAseq Beads to each sample. Mix by pipetting up and down 10 times and then briefly centrifuge.
3. Incubate for 5 min at room temperature.
4. Place the tubes/plate on a magnetic rack for 5 min. After the solution has cleared, carefully remove and discard the supernatant without disturbing the beads.

   Note: Keep the beads on the magnetic stand while handling the supernatant and during the washing steps.

   Important: Do not discard the beads as they contain the DNA of interest.
5. Add 200 µL 80% ethanol while it is on the magnetic rack/plate. Rotate the tube (3 times) or move the plate side to side in the 2 positions of the magnet to wash the beads. Carefully remove and discard the wash.
6. Repeat the ethanol wash in step 5.

   Important: Completely remove all traces of ethanol after the second wash. To do this, briefly centrifuge (1 s) and return the tubes or plate to the magnetic stand. Remove the ethanol first with a 200 µL pipette, and then use a 10 µL pipette to remove any residual ethanol.
7. With the tubes/plate still on the magnet, air-dry at room temperature for 2–5 min (up to 15 min when using a plate).

Note: Visually inspect that the pellet is dry. When dry, the beads should lose its shiny appearance. Overdrying of beads may result in lower DNA recovery.

8. Elute the DNA by resuspending in 18.25 µL Nuclease-Free Water. Remove tubes from magnetic rack and mix well by pipetting. Incubate for 1–2 min at room temperature. Place a tube or plate on the magnetic rack until the solution is clear.
9. Transfer 15.75 µL of the supernatant to a new PCR tube or plate.

Protocol: QIAseq FX DNA Library Kit

The reagents required for this protocol are found in the QIAseq FX DNA Library Kit. This protocol describes the FX reaction for single-tube fragmentation, end-repair, and A-addition.

If sample only contains DNA, then the protocol can start with QIAseq FX DNA Library Kit (Table 8).

Important points before starting

- Ensure input DNA is in water, 10 mM Tris, or QIAGEN's Buffer EB. If input DNA is in 1x TE, please remove EDTA from the DNA by following the protocol in Appendix A.
- Ensure reactions are thoroughly mixed, as well as prepared and incubated at the recommended temperatures.
- Pre-program thermal cyclers. For increased speed and convenience, all incubation steps of the protocol can be preprogrammed and saved in advanced. Additionally, the program can be started and paused while setting-up the reaction.

Fragmentation, end-repair, and A-addition

Procedure

1. Thaw reagents on ice. Once reagents are thawed, mix buffers thoroughly by quick vortexing to avoid any localized concentrations. Briefly spin down vortexed reagents before use.
2. Program a thermal cycler according to Table 8 and pause the program at the 4°C step.

Table 8. Fragmentation reaction

Temperature (°C)	Time
4	1 min
32	16 min
65	30 min
4	Hold

3. On ice, add the following components in Table 9 to each cleaned-up second strand product. Mix well by pipetting up and down 15 times and spin down.

Table 9. Fragmentation reaction setup for samples from second strand synthesis

Component	Per sample (µL)
Cleaned up second strand product	15.75
FX Buffer, 10x	2.25
Total volume	**18**

Table 10. Alternative fragmentation setup

Component	Per sample (µL)
Microbial DNA sample	x
FX Buffer, 10x	2.25
Nuclease-Free Water	15.75–x
Total volume	**18**

Fragmentation reaction setup for samples that only contain DNA and did not undergo Protocol: Converting RNA to Double-Stranded cDNA.

4. Add 4.5 µL FX Enzyme Mix to each reaction and mix well by pipetting up and down 15 times. It is critical to keep the reactions on ice during the reaction setup.
5. Briefly spin down the PCR tubes/plate and immediately transfer to the pre-chilled thermal cycler (4°C). Resume the cycling program.

6. When the thermal cycler is complete and the sample block has returned to 4°C, remove the samples and immediately place them on ice.

Adapter ligation

Notes before starting

- UPH Ligation Buffer replaces DNA Ligation Buffer, 5x, that is supplied in the QIAseq FX Library Kit.
- The UDI adapter barcode sequences used in the QIAseq FX DNA Library Kit 96-plex adapter plate, as well as the layout of the 96-plex and 24-plex single use adapter plates, are described in Appendix C.
- 80% ethanol should be freshly prepared using Nuclease-Free Water and mixed thoroughly by vortexing.
- Before cleanup, ensure that the QIAseq Beads have been equilibrated to room temperature for 30 minutes.
- **Important**: QIAseq Beads need to be homogenous. Thoroughly resuspend the beads immediately before use and process the beads quickly. If a delay in the protocol occurs, simply vortex the beads again.
- Pre-program thermal cyclers. For increased speed and convenience, all incubation steps of the protocol can be preprogrammed and saved in advanced. Additionally, the program can be started and paused while setting-up the reaction.

Procedure

1. Remove the protective adapter plate lid, pierce the foil seal for each adapter well to be used, and transfer 2.5 µL from one DNA adapter well to each 50 µL sample from the previous protocol. Track the barcodes from each adapter well used for each sample.
2. Replace the adapter plate lid and freeze unused adapters. The adapter plate is stable for a minimum of 10 freeze–thaw cycles.

Important: Only 1 single adapter is to be used per ligation reaction. Do not reuse adapter wells once the foil seal has been pierced.

3. Prepare ligation reaction following Table 11. On ice, add the following components to each sample. Mix well by pipetting up and down 15 times and spin down.

Table 11. Ligation setup

Component	Per sample (µL)
DNA Adapter	2.5
UPH Ligation Buffer*	20
DNA ligase	5
Fragmentation reaction	22.5
Total volume	**50**

* This replaces the DNA Ligation Buffer, 5x, from the QIAseq FX kit.

4. Incubate the reactions in a thermal cycler at 20°C for 15 min.

   Important: Do not use heated lid.

5. Proceed immediately to adapter ligation cleanup.

Cleanup after adapter ligation with QIAseq Beads

Procedure

1. Add 50 µL Nuclease-free Water to the ligation reaction.
2. Add 90 µL (0.9x) QIAseq Beads to each sample. Mix by pipetting up and down 10 times and then briefly centrifuge.
3. Incubate for 5 min at room temperature.
4. Place the tubes/plate on a magnetic rack for 5 min. After the solution has cleared, carefully remove and discard the supernatant without disturbing the beads.

Note: Keep the beads on the magnetic stand while handling the supernatant and during the washing steps.

Important: Do not discard the beads as they contain the DNA of interest.

5. Add 200 µL 80% ethanol while it is on the magnetic rack/plate. Rotate the tube (3 times) or move the plate side to side in the 2 positions of the magnet to wash the beads. Carefully remove and discard the wash.
6. Repeat the ethanol wash in step 5.

   Important: Completely remove all traces of ethanol after the second wash. To do this, briefly centrifuge (1 s) and return the tubes or plate to the magnetic stand. Remove the ethanol first with a 200 µL pipette, and then use a 10 µL pipette to remove any residual ethanol.
7. With the tubes/plate still on the magnet, air-dry at room temperature for 2–5 min (up to 15 min when using a plate).

   Note: Visually inspect that the pellet is dry. When dry, the beads should lose its shiny appearance. Overdrying of beads may result in lower DNA recovery.
8. Elute the DNA by resuspending in 52.5 µL Nuclease-Free Water. Remove tubes from magnetic rack and mix well by pipetting. Incubate for 1–2 min at room temperature. Place a tube or plate on the magnetic rack until the solution is clear.
9. Transfer 50 µL of the supernatant to a new PCR tube or plate.
10. Add 55 µL (1.1x) QIAseq Beads to each sample. Mix by pipetting up and down 10 times and then briefly centrifuge.
11. Incubate for 5 min at room temperature.
12. Place the tubes/plate on a magnetic rack for 5 min. After the solution has cleared, carefully remove and discard the supernatant without disturbing the beads.

    Note: Keep the beads on the magnetic stand while handling the supernatant and during the washing steps.

    Important: Do not discard the beads as they contain the DNA of interest.

13. Add 200 µL 80% ethanol while it is on the magnetic rack/plate. Rotate the tube (3 times) or move the plate side to side in the 2 positions of the magnet to wash the beads. Carefully remove and discard the wash.
14. Repeat the ethanol wash in step 13.

    Important: Completely remove all traces of ethanol after the second wash. To do this, briefly centrifuge (1 s) and return the tubes or plate to the magnetic stand. Remove the ethanol first with a 200 µL pipette, and then use a 10 µL pipette to remove any residual ethanol.
15. With the tubes/plate still on the magnet, air-dry at room temperature for 2–5 min (up to 15 min when using a plate).

    Note: Visually inspect that the pellet is dry. When dry, the beads should lose its shiny appearance. Overdrying of beads may result in lower DNA recovery.
16. Elute the DNA by resuspending in 25 µL Nuclease-Free Water. Remove tubes from magnetic rack and mix well by pipetting. Incubate for 1–2 min at room temperature. Place a tube or plate on the magnetic rack until solution is clear.
17. Transfer 22.5 µL of the supernatant to a new PCR tube or plate.

Library amplification

Notes before starting

- Thaw QIAseq HiFi PCR Master Mix, Primer Mix, and Lib Amp Blocker on ice. Once reagents are thawed, mix them thoroughly by quick vortexing to avoid any localized concentrations.
- Always start with the cycling conditions specified in this protocol. The cycling has been optimized for use with QIAseq HiFi PCR Master Mix for even and high-fidelity amplification of sequencing libraries.
- 80% ethanol should be freshly prepared using Nuclease-Free Water and mixed thoroughly by vortexing.

- Before cleanup, ensure that the QIAseq Beads have been equilibrated to room temperature for 30 minutes.
- **Important**: QIAseq Beads need to be homogenous. Thoroughly resuspend the beads immediately before use and process the beads quickly. If a delay in the protocol occurs, simply vortex the beads again.
- Pre-program thermal cyclers. For increased speed and convenience, all incubation steps of the protocol can be preprogrammed and saved in advanced. Additionally, the program can be started and paused while setting-up the reaction.

Procedure

1. Prepare a reaction mix according to Table 12. On ice, add the following components to each sample. Mix well by pipetting up and down 10 times and spin down.

Table 12. Library amplification setup

Component	Per sample (µL)
HiFi PCR Master Mix, 2x	25
Primer mix	1.5
Lib Amp Blocker	1
Library DNA	22.5
Total volume	**50**

2. Transfer the PCR tube or plate to the thermocycler and start the program according to Table 13.

Table 13. Library amplification cycling conditions

Step	Time	Temperature (°C)
Hold	2 min	98
3 step cycling		
Step 1	20 s	98
Step 2	30 s	60
Step 3	30 s	72
14 cycles*		
Final Extension (1 cycle)	1 min	72
Hold	∞	4

* A total of 14 cycles is recommended when starting as it will yield sufficient library for hybrid capture pooling for most sample types. However, this can be adjusted depending on initial input amount. For example, if overamplification is a concern, then the number of cycles can be reduced. Alternatively, if not enough library is generated for pooling, then the number of cycles can be increased.

Cleanup after library amplification with QIAseq Beads

1. Add 55 µL (1.1x) QIAseq Beads to each sample. Mix by pipetting up and down 10 times and then briefly centrifuge.
2. Incubate for 5 min at room temperature.
3. Place the tubes/plate on a magnetic rack for 5 min. After the solution has cleared, carefully remove and discard the supernatant without disturbing the beads.

   Note: Keep the beads on the magnetic stand while handling the supernatant and during the washing steps.

   Important: Do not discard the beads as they contain the DNA of interest.
4. Add 200 µL 80% ethanol while it is on the magnetic rack/plate. Rotate the tube (3 times) or move the plate side to side in the 2 positions of the magnet to wash the beads. Carefully remove and discard the wash.
5. Repeat the ethanol wash in step 4.

Important: Completely remove all traces of ethanol after the second wash. To do this, briefly centrifuge (1 s) and return the tubes or plate to the magnetic stand. Remove the ethanol first with a 200 µL pipette, and then, use a 10 µL pipette to remove any residual ethanol.

6. With the tubes/plate still on the magnet, air-dry at room temperature for 2–5 min (up to 15 min when using a plate).

   Note: Visually inspect that the pellet is dry. When dry, the beads should lose its shiny appearance. Overdrying of beads may result in lower DNA recovery.

7. Elute the DNA by resuspending in 27.5 µL Nuclease-Free Water. Remove tubes from magnetic rack and mix well by pipetting. Incubate for 1–2 min at room temperature.
8. Transfer 25 µL of the supernatant to a new PCR tube or plate.
9. Assess the quality of the library using a capillary electrophoresis device such as QIAGEN QIAxcel® or Agilent Bioanalyzer. If the libraries are overamplified, then a spectrophotometric (QIAxpert® or Nanodrop) measurement is recommended to determine library concentration.
10. Proceed to "Protocol: Hybrid Capture and Wash". Alternatively, the completed libraries can be stored at –30 to –15°C in a constant-temperature freezer.

Protocol: Hybrid Capture and Wash

Pool libraries and overnight hyb capture reaction

Important points before starting

- Ensure reactions are thoroughly mixed, as well as prepared and incubated at the recommended temperatures.
- Pre-program thermal cyclers. For increased speed and convenience, all incubation steps of the protocol can be preprogrammed and saved in advanced. Additionally, the program can be started and paused while setting up the reaction.
- Thaw the QIAseq xHYB Probe Set, Enhanced Blocking Buffer, One-4-All Blocking Oligos, and One-4-All Blocking Solution on ice, and then, pulse vortex and pulse-spin.
- To immediately proceed to hybridization after library pool dry-down, equilibrate the Vapor-Lock reagent to room temperature, and heat the Hybridization Solution to 65°C for 10 min or until all precipitate is dissolved, before pool evaporation is complete. Alternatively, the dried library pool can be stored at –30 to –15°C for up to 3 days.
- For each hyb capture pool, a QIAseq probe panel/hybridization mix are prepared in a 1.5 mL LoBind tube. For each each hyb capture pool, the dried-down libraries/One-4-All Blocking Solution/One-4-All Blocking Oligos are prepared in a separate tube. After both tubes are finished incubating at RT/23°C, they are combined.
- While a Speed-Vac is recommended to dry down the libraries, if one is not available, then follow Appendix B: Replacing Speed-Vac Concentration with QIAseq Beads to concentrate the libraries before hybridization.

Procedure

1. Add 375 ng of each library (4 libraries per pool) for a total of 1500 ng per pool to a 1.5 mL LoBind tube. Add 3.5 µL of Enhanced Blocking Buffer per pool. Use multiple tubes if performing multiple hybridization reactions.

Note: If total library yield is less than 375 ng, then it is possible to decrease amount of library to a minimum of 125 ng per library. It is recommended to add equal amounts of library in a pool. Decreasing input amount may lead to lower diversity.

2. Completely evaporate all liquid content of the library pool by using a SpeedVac system (or a similar evaporator device). If needed, accelerate the evaporation of larger volumes by setting the temperature to 60°C.

   Note: Proceed to Appendix B if performing library pool concentration via QIAseq Beads.

3. When evaporation of the library pool is complete, place the dried library pool on ice and proceed to step 4 (Hybridization Mix/QIAseq probe panel). Alternatively, the dried library pool can be stored at –30 to –15°C for up to 3 days.
4. If not previously done so, heat the Hybridization Mix to 65°C for 10 min or until precipitate is dissolved, then let cool to RT for 5 min. In addition, equilibrate Vapor-Lock to room temperature.
5. For each library pool, prepare the Hybridization Mix/QIAseq probe panel in a separate PCR tube by following Table 14.

Table 14. QIAseq probe prep

Component	Per sample (µL)
Hybridization Mix	20
QIAseq probe panel	4
Nuclease-Free Water or QIAseq probe spike-in*	4
Total volume	**28**

*Nuclease-Free Water can be replaced with a QIAseq probe spike-in panel, for example, MPXV spike-in Panel.

6. Incubate the Hybridization Mix/QIAseq probe panel in a thermal cycler by following Table 15.

Table 15. QIAseq probe prep reaction

Temperature	Time (min)
95°C	2
On ice	5
RT/23°C	5

7. While the Hybridization Mix/QIAseq probe prep is cooling, completely resuspend the dried-down libraries from step 3 by adding components according to Table 16. Mix well by pipetting up and down and/or vortex and briefly centrifuge.

   Note: This step should be performed concurrently (or as close as possible) with steps 5 and 6. Alternatively, resuspension of the dried-down libraries can be performed as soon as evaporation of the libraries is complete (step 3) then placed on ice until needed.

Table 16. Resuspension of dried-down library

Component	Per sample (µL)
Dried-down libraries	–
One-4-All Blocking Solution	5
One-4-All Blocking Oligos	8
Total volume	**13**

8. Transfer contents to a new PCR tube.
9. Incubate the resuspended libraries in a thermal cycler by following Table 17.

Table 17. Blocking of Dried-down library reaction

Temperature (°C)	Time (min)
95	5
RT/23	<5

10. Carefully mix Hybridization Mix/QIAseq Probe Panel solution (from step 6) by pipetting up and down then add entire contents to resuspended library (from step 9).
11. Add 30 µL of Vapor-Lock and carefully mix by gently pipetting up and down. Briefly spin down.
12. Incubate hybridization reaction at 70°C for 16 h in a thermal cycler with heated lid at 85°C.

Preparing streptavidin beads

Important points before starting

- Inspect Binding Buffer, Wash Buffer 1, and Wash Buffer 2 for any precipitate.
- If precipitate is observed, heat buffer at 48°C until all precipitate is dissolved into solution.
- Prepare 450 µL of Wash Buffer 1 for each Hybridization Reaction and preheat to 60°C.
- Prepare 650 µL of Wash Buffer 2 for each Hybridization Reaction, and preheat to 48°C.
- Equilibrate Streptavidin Binding Beads and DNA Purification Beads to room temperature for at least 30 min.

Procedure

1. For each hybridization reaction, add 100 µL of Streptavidin Binding Beads to a clean 1.5 mL LoBind tube.
2. For each hybridization reaction, add 200 µL of Binding Buffer to the tube and mix by pipetting.
3. Place tube on magnetic stand for 1 min. Discard supernatant.
4. Repeat wash (steps 2 and 3) two additional times.
5. Add 200 µL of Binding Buffer to the tube and resuspend by vortexing.

Binding and washing of hybridized targets to streptavidin beads

1. After hybridization reaction is complete and while still on thermal cycler at 70°C, swiftly transfer full volume of hybridization reaction including Vapor-Lock to corresponding tube of streptavidin beads. Mix well by pipetting up and down.

   Important: Do not let hybridization reaction cool before transferring to streptavidin beads.

2. Mix tube containing hybridization reaction and streptavidin beads for 30 min at RT on a shaker, rocker, or rotator so that the solution remains homogenized.

   Note: Aggressive mixing is not required. Do not vortex.

3. Briefly centrifuge the tube to collect all the liquid to the bottom of the tube.
4. Place the beads on a magnetic stand for 1 min or until solution is clear. Carefully discard supernatant with the Vapor-Lock without disturbing the beads.
5. Remove the tube from the magnetic stand and add 200 µL of preheated 60°C Wash Buffer 1. Mix by pipetting up and down and briefly centrifuge. Transfer contents to a new 1.5 mL LoBind tube.

   Note: Place Wash Buffer 1 back at 60°C until needed for further washes.

6. Place the tube on a magnetic stand for 1 min or until the solution is clear. Discard the clear supernatant without disturbing the pellet.
7. Repeat wash 1 (steps 5 to 7) one time.
8. Remove the tube from the magnetic stand and add 200 µL of preheated 48°C Wash Buffer 2. Mix by pipetting up and down and briefly centrifuge.
9. Incubate at 48°C for 5 min. Pulse spin down.

   Note: Place Wash Buffer 2 back at 48°C until needed for further washes.

10. Place the tube on the magnetic stand for 1 min. Discard the clear supernatant without disturbing the pellet.
11. Repeat wash (steps 8–11) two additional times for total of three washes.

12. Briefly centrifuge the tube and remove all remaining liquid using a 10 µL pipette tip without disturbing the beads.

    Important: Do not allow the beads to dry.

13. Immediately add 50 µL Hyb Elute Buffer and mix by pipetting up and down.
14. Transfer 23.5 µL of Hyb Elute Buffer bead slurry to a new PCR tube.
15. Proceed directly to "Post-capture library amplification". Alternatively, the resuspended Hyb Elute Buffer bead slurry can be stored at –30 to –15°C in a constant temperature freezer-.

Post-capture library amplification

Important points before starting

- 80% ethanol should be freshly prepared using Nuclease-Free Water and mixed thoroughly by vortexing.
- Before cleanup, ensure that the QIAseq Beads have been equilibrated to room temperature for 30 minutes.
- **Important**: QIAseq Beads need to be homogenous. Thoroughly resuspend the beads immediately before use and process the beads quickly. If a delay in the protocol occurs, simply vortex the beads again.
- Preprogram thermal cyclers. For increased speed and convenience, all incubation steps of the protocol can be preprogrammed and saved in advanced. Additionally, the program can be started and paused while setting up the reaction.
- Thaw Post Hybrid-Capture PCR Mix and Primer Mix Illumina Library Amplification on ice. Once reagents are thawed, mix by pulse-vortexing to avoid any localized concentrations. Briefly spin down vortexed reagents before use.

Procedure

1. Prepare a reaction mix according to Table 18. On ice, add the following components to each sample. Mix well by pipetting up and down 10 times and spin down.

Table 18. Post Hybrid-Capture Amplification

Component	Per sample (µL)
Post Hybrid-Capture PCR Mix 2x	25
Primer mix	1.5
Hyb Elute Buffer bead slurry*	23.5*
Total volume	**50**

* Save the remaining Hyb Elute Buffer bead slurry in case the post-capture amplification needs to be repeated.

2. Perform library amplification in a thermal cycler by following Table 19.

Table 19. Post Hybrid-Capture amplification cycling conditions

Step	Time	Temperature (°C)
Hold	2 min	98
3 step cycling		
Step 1	20 s	98
Step 2	30 s	60
Step 3	30 s	72
20 cycles*		
Final Extension (1 cycle)	1 min	72
Hold	∞	4

* For most hybrid capture pools, 20 cycles will generate sufficient library. There is sufficient Hyb Elute Buffer bead slurry to perform an additional Post Hybrid-Capture amplification if there is no sufficient library for sequencing (suggest 23–25 cycles) or if there is extensive overamplification (suggest 14–17 cycles).

Cleanup of library amplification with QIAseq Beads

Procedure

1. Add 55 µL (1.1x) QIAseq Beads to each sample. Mix by pipetting up and down 10 times and then briefly centrifuge.
2. Incubate for 5 min at room temperature.

3. Place the tubes/plate on a magnetic rack for 5 min. After the solution has cleared, carefully remove and discard the supernatant without disturbing the beads.

   Note: Keep the beads on the magnetic stand while handling the supernatant and during the washing steps.

   Important: Do not discard the beads as they contain the DNA of interest.

4. Add 200 µL 80% ethanol while it is on the magnetic rack/plate. Rotate the tube (3 times) or move the plate side to side in the 2 positions of the magnet to wash the beads. Carefully remove and discard the wash.

5. Repeat the ethanol wash in step 4.

   Important: Completely remove all traces of ethanol after the second wash. To do this, briefly centrifuge (1 s) and return the tubes or plate to the magnetic stand. Remove the ethanol first with a 200 µL pipette, and then use a 10 µL pipette to remove any residual ethanol.

6. With the tubes/plate still on the magnet, air-dry at room temperature for 2–5 min (up to 15 min when using a plate).

   Note: Visually inspect that the pellet is dry. When dry, the beads should lose its shiny appearance. Overdrying of beads may result in lower DNA recovery.

7. Elute the DNA by resuspending in 25 µL Nuclease-Free Water. Remove tubes from magnetic rack and mix well by pipetting. Incubate for 1–2 min at room temperature.

8. Transfer 22.5 µL of the supernatant to a new PCR tube or plate.

9. Proceed to "Protocol: Library QC and Quantification". Alternatively, the completed libraries can be stored at –30 to –15°C in a constant-temperature freezer.

Protocol: Library QC and Quantification

This protocol determines the quality and quantity of each xHYB library.

Important points before starting

- A portion of either the xHYB post-capture library is the starting material for the library QC and quantification. When not in use, the xHYB post-capture library should be stored on ice.
- Library QC involves use of an Agilent® 2100 Bioanalyzer, TapeStation, or similar capillary electrophoresis device.
- Library quantification involves use of QIAGEN's QIAseq Library Quant System: QIAseq Library Quant Array Kit (cat. no. 333304) or QIAseq Library Quant Assay Kit (cat. no. 333314).

Library QC (capillary electrophoresis)

1. Analyze 1 µL of the xHYB post-capture library on a capillary electrophoresis device according to the manufacturer's instructions. A typical xHYB post-capture library is shown in Figure 2 (see next page).
2. Proceed to "Determining Library Concentration", page 41.

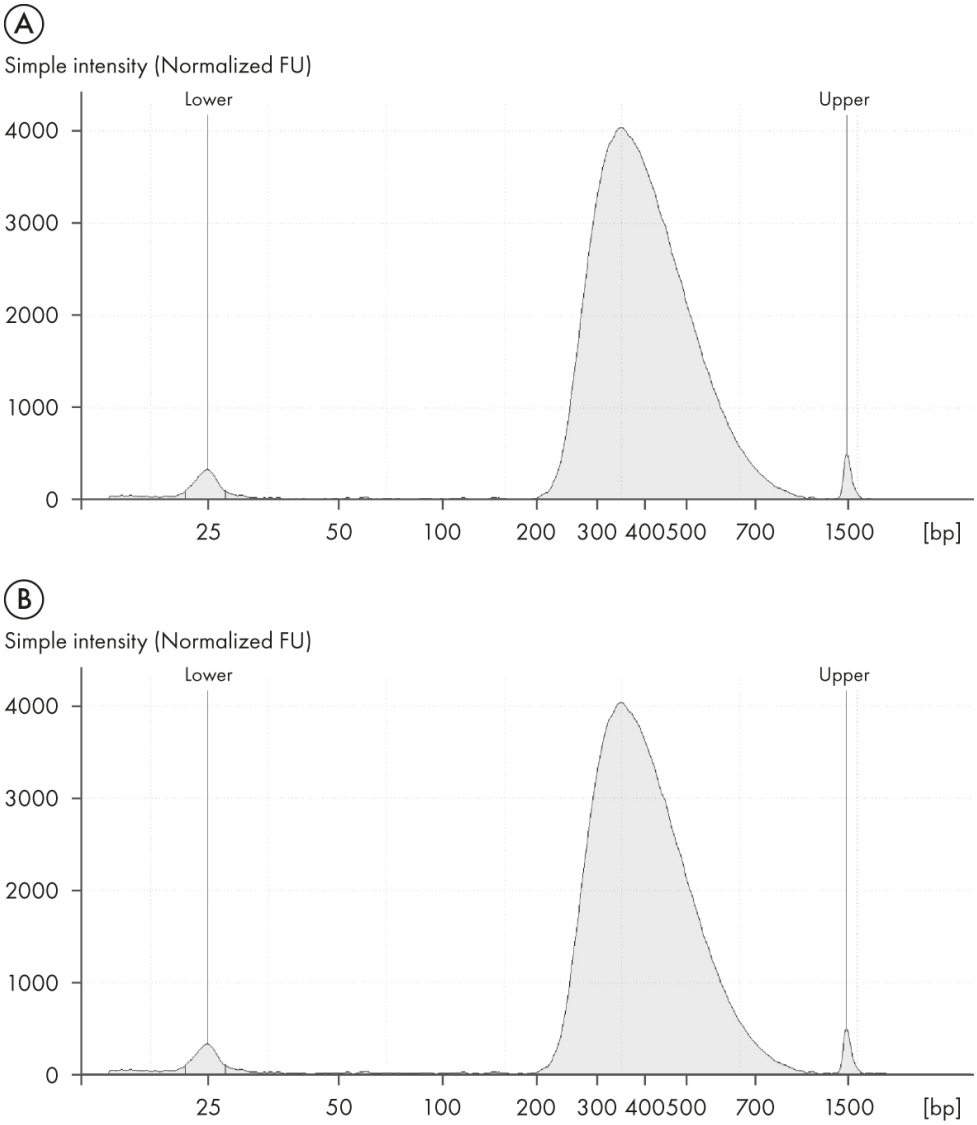

Figure 2. Tapestation Trace of a library prepared with QIAseq xHYB. (A) An example RNA/TNA/DNA library with a typical mean size distribution of 350–450 bp and free of adapter dimers. **(B)** An example of a post-capture library pool with a similar size distribution as the original libraries.

Determining library concentration

1. The library yield measurements of the Bioanalyzer or TapeStation system use fluorescent dyes that intercalate into DNA or RNA and cannot discriminate between DNA with or without adapter sequences. Real-time PCR-based methods provide an accurate quantification of complete QIAseq xHYB post-capture libraries with full adapter sequences. Therefore, QIAGEN's QIAseq Library Quant Array Kit or Assay Kit, which contains laboratory-verified forward and reverse primers together with a DNA standard, is highly recommended for accurate quantification of the prepared library.

   For the denaturation procedure to load the MiSeq sequencing instrument, 4 nM of QIAseq xHYB post-capture library should be used as input.

2. Proceed with "Protocol: Sequencing Setup on Illumina Instrument", next page.

Protocol: Sequencing Setup on Illumina Instrument

Important points before starting

- **Important**: Recommendations for library dilution concentrations and library loading concentrations are based on QIAGEN's QIAseq Library Quant System (see "Determining library concentration", page 41).
- **Important**: Paired-end sequencing should be used for the QIAseq xHYB on an Illumina platform.
- Please refer to the system-specific Illumina documents for complete instructions on how to denature sequencing libraries, prepare phiX, and set up a sequencing run.

Pooling guidelines

Important: As a starting point, we recommend allocating 1M clusters per original sample or 4M clusters per hybrid capture pool (assuming 4 libraries per hybrid capture pool). Table 20 describes the number of samples that can be multiplexed. Number of reads per sample may need to be increased depending on pooling of samples with high variation in viral load, and/or sample type; that is, wastewater may require more reads/sample. We recommend 5 – 10 million read clusters per sample as a starting point for wastewater samples. Final read allocation and sequencing performance will depend on viral load, enriched viral genome length, any co-infections, etc.

Table 20. Sample multiplexing suggestions

Instrument	Version	Clusters/flow cell (millions)	Samples/flow cell*
iSeq 100	i1 Reagents	4	4
MiniSeq	Mid Output	8	8
MiniSeq	High Output	25	24
MiSeq	v2 Reagents	15	14
MiSeq	v3 Reagents	25	24
NextSeq 500/550	Mid Output	130	120
NextSeq 500/550	High Output	400	360
HiSeq 1500/2500	Rapid Run v2 with cBot	150 (per lane)	135
HiSeq 3000/4000	–	312.5 (per lane)	281
NovaSeq 6000	SP	800	720
NovaSeq 6000	S1	1600	1440

* For wastewater, we recommend 5 – 10 million read clusters per sample as a starting point.

Sequencing

- **Important**: The following guidelines outline the most important settings for Illumina instruments. More detailed instructions on how to configure a run and how to create a sample sheet can be found in the "Product Resources" section for QIAseq Y-Adapters at **www.qiagen.com.**
- Always ensure that libraries have been quantified using QIAseq Library Quant Assay or a compatible method to enable equal library representation within the sequencing pool and exact pool concentrations for optimal flow cell loading and best sequencing performance.
- For complete instructions on how to denature sequencing libraries and set up a sequencing run, please refer to the system-specific Illumina documents.
- Please refer to the respective *QIAseq FX DNA Library Handbook* for detailed information on Unique Dual-Index Adapters.

- Editable and ready to use sample sheets, including all sample indexes for UDI Y-Adapters, are available for download at **www.qiagen.com**.
- A description of run setup for Illumina instruments and definition files for QIAseq UDI-Y adapters to be used for Illumina Experiment Manager and Local Run Manager are available at **www.qiagen.com**.
- Sequencing on the NextSeq, HiSeq X®, HiSeq 3000/4000 systems follow a dual-indexing workflow different from other Illumina systems. If manually creating sample sheets on these instruments, enter the reverse complement of the i5 index adapter sequence. If using Illumina Experiment Manager, BaseSpace®, or Local Run Manager to plan the run, the software will automatically reverse complement index sequences when necessary.

Sequencing setup on Illumina instruments

- Read Type: Paired End
- Index Reads: 2
- Enable Adapter Trimming
- Cycles for QIAseq UDI Y-Adapters
- Read 1 and Read 2: 149
- Index 1 and Index 2: 10

Protocol: Data Analysis

Important points before starting

- Register and sign-in to GeneGlobe data analysis center: **https://geneglobe.qiagen.com/us/analyze**.
- Depending on the panel, GeneGlobe data analysis provides both Viral Sequencing Analysis or Antimicrobial Resistance Analysis.
- For Viral Sequencing Analysis, GeneGlobe outputs include merged abundance tables based on taxonomy, combined sequencing quality report, low coverage area, ***.vcf** files, and virus consensus sequence. Additional files such as amino acid track, annotated variant track, and read mapping are outputted to be used in CLC Genomics Workbench.
- GeneGlobe Antimicrobial Resistance Analysis is based on the "Find Resistance with ShortBRED" tool in CLC Genomics Workbench. GeneGlobe outputs include resistance tables and combined sequencing quality report.
- For further and more detailed analysis, CLC Genomics Workbench is highly recommended.

GeneGlobe data analysis

1. Go to GeneGlobe data analysis center: **https://geneglobe.qiagen.com/us/analyze**.
2. Under each section, select the following:
   - Select analysis type: Next-Generation Sequencing
   - Select your analyte: Microbial
   - Select your panel: QIAseq xHYB
3. Press the **Start Your Analysis** button.
4. Upload ***.fastq** files either locally or transfer from BaseSpace.
5. Once ***.fastq** files are uploaded, select files for analysis and press the **Select For Analysis** button.

6. Fill out the “Job Title” field.
7. For QIAseq xHYB Respiratory, QIAseq xHYB Viral STI, and QIAseq xHYB Adventitious Agent Panels, select “Viral Sequencing Analysis” for analysis mode. For QIAseq xHYB AMR Panel, select “Antimicrobial Resistance Analysis” for analysis mode.
8. Press the **Analyze** button.
9. After analysis is completed, reports can be downloaded to be viewed offline.

Troubleshooting Guide

This troubleshooting guide may be helpful in solving any problems that may arise. For more information, see also the Frequently Asked Questions page in our Technical Support Center: **www.qiagen.com/FAQ/FAQList.aspx**. The scientists in QIAGEN Technical Services are always happy to answer any questions you may have about either the information or protocols in this handbook (for contact information, visit **support.qiagen.com**).

	Comments and suggestions
Low library yield	
a) DNA contains PCR inhibitors carried over from sample prep	If samples contain high concentration of PCR inhibitors (such as stool, soil, or wastewater), use the appropriate sample DNA extraction kit.
b) Improper reaction setup	Ensure reactions are thoroughly mixed, prepared, and incubated at recommended temperatures.
c) Excess ethanol not removed during bead cleanup steps	For each cleanup, after final ethanol wash, ensure that excess ethanol is removed. Briefly centrifuge and return the tubes or plate to the magnetic stand. Remove with a 10 µL pipette to remove any residual ethanol. In addition, allow beads to dry for the appropriate amount of time.
Low hybrid capture library yield	
a) Insufficient sample material added to library construction	If possible, increase sample input in library construction step.
b) Incompatible adapters used	Using incompatible non–TruSeq-like adapters for the generation of whole genome libraries will result in failure to amplify captured library fragments. Use only UDI Y-Adapters or other TruSeq-compatible adapters for the generation of indexed libraries.

	Comments and suggestions
Sequencing issues	
a) Too low or too high cluster density	Accurate library quantification is the key for optimal cluster density on any sequencing instrument. PCR-based quantification method is recommended. Other methods may lead to the incorrect quantification of the library especially when there is over-amplification.
b) Very low clusters passing filter	Make sure the library is accurately quantified and that the correct amount is loaded onto the sequencing instrument.
Unequal read distribution across libraries in the same hybrid capture pool	
Viral loads may be significantly different between samples.	If qPCR data are available, then pool samples with similar Ct values (~within 3–4 Ct).

Appendix A: Cleanup of DNA samples that contain EDTA/EGTA

Important points before starting

- 80% ethanol should be freshly prepared using Nuclease-Free Water and mixed thoroughly by vortexing.
- Before cleanup, ensure that the QIAseq Beads have been equilibrated to room temperature for 30 min.
- **Important**: QIAseq Beads need to be homogenous. Thoroughly resuspend the beads immediately before use and process the beads quickly. If a delay in the protocol occurs, simply vortex the beads again.

Procedure

1. Add Nuclease-Free Water to DNA sample so that final volume equals 50 µL.
2. Add 90 µL (1.8x) QIAseq Beads to each sample. Mix by pipetting up and down 10 times and then briefly centrifuge.
3. Incubate for 5 min at room temperature.
4. Place the tubes/plate on a magnetic rack for 5 min. After the solution has cleared, carefully remove and discard the supernatant without disturbing the beads.

   Note: Keep the beads on the magnetic stand while handling the supernatant and during the washing steps.

   Important: Do not discard the beads as they contain the DNA of interest.
5. Add 200 µL 80% ethanol while it is on the magnetic rack/plate. Rotate the tube (3 times) or move the plate side to side in the 2 positions of the magnet to wash the beads. Carefully remove and discard the wash.

6. Repeat the ethanol wash in step 5.

   Important: Completely remove all traces of ethanol after the second wash. To do this, briefly centrifuge (1 s) and return the tubes or plate to the magnetic stand. Remove the ethanol first with a 200 µL pipette, and then use a 10 µL pipette to remove any residual ethanol.

7. With the tubes/plate still on the magnet, air-dry at room temperature for 2–5 min (up to 15 min when using a plate).

   Note: Visually inspect that the pellet is dry. When dry, the beads should lose its shiny appearance.

8. While still on the magnetic rack/plate, elute the DNA from the beads by adding 18.25 µL Nuclease-Free Water. Remove tubes from magnetic rack and mix well by pipetting. Incubate for 2–5 min at room temperature.

9. Transfer 15.75 µL of the supernatant to a new PCR tube or plate.

Appendix B: Replacing Speed-Vac Concentration with QIAseq Beads

Important points before starting

- **Note**: There may be a small reduction in performance (approx. 10% decrease in unique reads) compared to the Speed-Vac protocol.
- 80% ethanol should be freshly prepared using Nuclease-Free Water and mixed thoroughly by vortexing.
- Before cleanup, ensure that the QIAseq Beads have been equilibrated to room temperature for 30 min.
- **Important**: QIAseq Beads need to be homogenous. Thoroughly resuspend the beads immediately before use and process the beads quickly. If a delay in the protocol occurs, simply vortex the beads again.
- For each hyb capture, combine 5.15 µL One-4-All Blocking Solution + 3.60 µL of Enhanced Blocking Buffer to make QIAseq bead elution buffer.

Procedure

1. Add 450 ng of each library (4 libraries per pool) for a total of 1800 ng per pool to a 1.5 mL LoBind tube. Use multiple tubes if performing multiple hybridization reactions.
2. Add Nuclease-Free Water so that final volume of each pool is 50 µL.
3. Add 90 µL (1.8x) QIAseq Beads to each sample. Mix by pipetting up and down 10 times and then briefly centrifuge.
4. Incubate for 5 min at room temperature.

5. Place the tubes on a magnetic rack for 5 min. After the solution has cleared, carefully remove and discard the supernatant without disturbing the beads.

   Note: Keep the beads on the magnetic stand while handling the supernatant and during the washing steps.

   Important: Do not discard the beads as they contain the DNA of interest.

6. Add 200 µL 80% ethanol while it is on the magnetic rack/plate. Rotate the tube (3 times) or move the plate side to side in the 2 positions of the magnet to wash the beads. Carefully remove and discard the wash.
7. Repeat the ethanol wash in step 4.

   Important: Completely remove all traces of ethanol after the second wash. To do this, briefly centrifuge (1 s) and return the tubes or plate to the magnetic stand. Remove the ethanol first with a 200 µL pipette, and then use a 10 µL pipette to remove any residual ethanol.

8. With the tubes/plate still on the magnet, air-dry at room temperature for 2–5 min (up to 15 min when using a plate).

   Note: Visually inspect that the pellet is dry. When dry, the beads should lose its shiny appearance.

9. While still on the magnetic rack/plate, elute the DNA from the beads by adding 7.5 µL QIAseq bead elution buffer + 8 µL of One-4-All Blocking Oligos. Mix well by pipetting. Incubate for 2–5 min at room temperature.
10. Place tubes on magnetic rack and wait 1 min. After the solution is cleared, carefully transfer 13 µL supernatant to a new tube without disturbing beads.
11. Place tubes on magnetic rack and wait 1 min. Confirm that there are no beads present in the solution.
12. Continue to “Protocol: Hybrid Capture and Wash”, step 8.

Appendix C: QIAseq Dual-Index Y-Adapters

Generation of sample sheets for Illumina instruments

Index sequences for QIAseq Unique and Combinatorial Dual-Index Y-Adapters are available for download at **www.qiagen.com**. Sequencing on the NextSeq, HiSeq X®, or HiSeq 3000/4000 system follows a dual-indexing workflow different from other Illumina systems. If you are manually creating sample sheets for these instruments, enter the reverse complement of the i5 index adapter sequence. If you are using Illumina Experiment Manager, BaseSpace, or Local Run Manager for run planning, the software will automatically reverse complement index sequences when necessary.

Ready-to-use sample sheets containing all QIAseq UDI Y-Adapter barcode sequences are available for MiSeq, NextSeq, MiniSeq, HiSeq, and NovaSeq instruments. These can be imported and edited using the Illumina Experiment Manager Software, Illumina Local Run Manager, or any text editor. Make sure to download the appropriate sample sheet for NextSeq, HiSeq X, or HiSeq 3000/4000 systems depending on whether you are using Local Run Manager or manually configuring the sequencing run.

Unique Dual-Index Y-Adapters

The layout of the 24-plex and 96-plex (A/B/C/D) single-use UDI adapter plate is shown in Figure 3 to Figure 7. The index sequences used in the QIAseq Unique Dual-Index Kits are listed in Table 21. To make sequencing preparation more convenient, you can download Illumina-compatible sample sheets for different sequencing instruments at **www.qiagen.com**.

	1	2	3	4	5	6	7	8	9	10	11	12
A	UDI 001	UDI 009	UDI 017	empty	empty	empty	empty	empty	empty	empty	empty	empty
B	UDI 002	UDI 010	UDI 018	empty	empty	empty	empty	empty	empty	empty	empty	empty
C	UDI 003	UDI 011	UDI 019	empty	empty	empty	empty	empty	empty	empty	empty	empty
D	UDI 004	UDI 012	UDI 020	empty	empty	empty	empty	empty	empty	empty	empty	empty
E	UDI 005	UDI 013	UDI 021	empty	empty	empty	empty	empty	empty	empty	empty	empty
F	UDI 006	UDI 014	UDI 022	empty	empty	empty	empty	empty	empty	empty	empty	empty
G	UDI 007	UDI 015	UDI 023	empty	empty	empty	empty	empty	empty	empty	empty	empty
H	UDI 008	UDI 016	UDI 024	empty	empty	empty	empty	empty	empty	empty	empty	empty

Figure 3. QIAseq UDI Y-Adapter Plate (24) layout (UDI 1–24).

	1	2	3	4	5	6	7	8	9	10	11	12
A	UDI 001	UDI 009	UDI 017	UDI 025	UDI 033	UDI 041	UDI 049	UDI 057	UDI 065	UDI 073	UDI 081	UDI 089
B	UDI 002	UDI 010	UDI 018	UDI 026	UDI 034	UDI 042	UDI 050	UDI 058	UDI 066	UDI 074	UDI 082	UDI 090
C	UDI 003	UDI 011	UDI 019	UDI 027	UDI 035	UDI 043	UDI 051	UDI 059	UDI 067	UDI 075	UDI 083	UDI 091
D	UDI 004	UDI 012	UDI 020	UDI 028	UDI 036	UDI 044	UDI 052	UDI 060	UDI 068	UDI 076	UDI 084	UDI 092
E	UDI 005	UDI 013	UDI 021	UDI 029	UDI 037	UDI 045	UDI 053	UDI 061	UDI 069	UDI 077	UDI 085	UDI 093
F	UDI 006	UDI 014	UDI 022	UDI 030	UDI 038	UDI 046	UDI 054	UDI 062	UDI 070	UDI 078	UDI 086	UDI 094
G	UDI 007	UDI 015	UDI 023	UDI 031	UDI 039	UDI 047	UDI 055	UDI 063	UDI 071	UDI 079	UDI 087	UDI 095
H	UDI 008	UDI 016	UDI 024	UDI 032	UDI 040	UDI 048	UDI 056	UDI 064	UDI 072	UDI 080	UDI 088	UDI 096

Figure 4. QIAseq UDI Y-Adapter Plate A (96) layout (UDI 1–96).

	1	2	3	4	5	6	7	8	9	10	11	12
A	UDI 097	UDI 105	UDI 113	UDI 121	UDI 129	UDI 137	UDI 145	UDI 153	UDI 161	UDI 169	UDI 177	UDI 185
B	UDI 098	UDI 106	UDI 114	UDI 122	UDI 130	UDI 138	UDI 146	UDI 154	UDI 162	UDI 170	UDI 178	UDI 186
C	UDI 099	UDI 107	UDI 115	UDI 123	UDI 131	UDI 139	UDI 147	UDI 155	UDI 163	UDI 171	UDI 179	UDI 187
D	UDI 100	UDI 108	UDI 116	UDI 124	UDI 132	UDI 140	UDI 148	UDI 156	UDI 164	UDI 172	UDI 180	UDI 188
E	UDI 101	UDI 109	UDI 117	UDI 125	UDI 133	UDI 141	UDI 149	UDI 157	UDI 165	UDI 173	UDI 181	UDI 189
F	UDI 102	UDI 110	UDI 118	UDI 126	UDI 134	UDI 142	UDI 150	UDI 158	UDI 166	UDI 174	UDI 182	UDI 190
G	UDI 103	UDI 111	UDI 119	UDI 127	UDI 135	UDI 143	UDI 151	UDI 159	UDI 167	UDI 175	UDI 183	UDI 191
H	UDI 104	UDI 112	UDI 120	UDI 128	UDI 136	UDI 144	UDI 152	UDI 160	UDI 168	UDI 176	UDI 184	UDI 192

Figure 5. QIAseq UDI Y-Adapter Plate B (96) layout (UDI 97–192).

	1	2	3	4	5	6	7	8	9	10	11	12
A	UDI 193	UDI 201	UDI 209	UDI 217	UDI 225	UDI 233	UDI 241	UDI 249	UDI 257	UDI 265	UDI 273	UDI 281
B	UDI 194	UDI 202	UDI 210	UDI 218	UDI 226	UDI 234	UDI 242	UDI 250	UDI 258	UDI 266	UDI 274	UDI 282
C	UDI 195	UDI 203	UDI 211	UDI 219	UDI 227	UDI 235	UDI 243	UDI 251	UDI 259	UDI 267	UDI 275	UDI 283
D	UDI 196	UDI 204	UDI 212	UDI 220	UDI 228	UDI 236	UDI 244	UDI 252	UDI 260	UDI 268	UDI 276	UDI 284
E	UDI 197	UDI 205	UDI 213	UDI 221	UDI 229	UDI 237	UDI 245	UDI 253	UDI 261	UDI 269	UDI 277	UDI 285
F	UDI 198	UDI 206	UDI 214	UDI 222	UDI 230	UDI 238	UDI 246	UDI 254	UDI 262	UDI 270	UDI 278	UDI 286
G	UDI 199	UDI 207	UDI 215	UDI 223	UDI 231	UDI 239	UDI 247	UDI 255	UDI 263	UDI 271	UDI 279	UDI 287
H	UDI 200	UDI 208	UDI 216	UDI 224	UDI 232	UDI 240	UDI 248	UDI 256	UDI 264	UDI 272	UDI 280	UDI 288

Figure 6. QIAseq UDI Y-Adapter Plate C (96) layout (UDI 193–288).

	1	2	3	4	5	6	7	8	9	10	11	12
A	UDI 289	UDI 297	UDI 305	UDI 313	UDI 321	UDI 329	UDI 337	UDI 345	UDI 353	UDI 361	UDI 369	UDI 377
B	UDI 290	UDI 298	UDI 306	UDI 314	UDI 322	UDI 330	UDI 338	UDI 346	UDI 354	UDI 362	UDI 370	UDI 378
C	UDI 291	UDI 299	UDI 307	UDI 315	UDI 323	UDI 331	UDI 339	UDI 347	UDI 355	UDI 363	UDI 371	UDI 379
D	UDI 292	UDI 300	UDI 308	UDI 316	UDI 324	UDI 332	UDI 340	UDI 348	UDI 356	UDI 364	UDI 372	UDI 380
E	UDI 293	UDI 301	UDI 309	UDI 317	UDI 325	UDI 333	UDI 341	UDI 349	UDI 357	UDI 365	UDI 373	UDI 381
F	UDI 294	UDI 302	UDI 310	UDI 318	UDI 326	UDI 334	UDI 342	UDI 350	UDI 358	UDI 366	UDI 374	UDI 382
G	UDI 295	UDI 303	UDI 311	UDI 319	UDI 327	UDI 335	UDI 343	UDI 351	UDI 359	UDI 367	UDI 375	UDI 383
H	UDI 296	UDI 304	UDI 312	UDI 320	UDI 328	UDI 336	UDI 344	UDI 352	UDI 360	UDI 368	UDI 376	UDI 384

Figure 7. QIAseq UDI Y-Adapter Plate D (96) layout (UDI 289–384).

Table 21. UDI index sequences used in the QIAseq UDI Y-Adapter Kits (24 and 96 A/B/C/D).

Unique Dual-Index adapters 1–24 are identical on the adapter plates of the QIAseq UDI Y-Adapter Kit (24) and QIAseq UDI Y-Adapter Kit A (96).

Note: Sequencing on the MiniSeq, NextSeq, HiSeqX, and HiSeq 3000/4000 systems follows a dual-indexing workflow different from other Illumina systems, which requires the reverse complement of the i5 index adapter sequence.

	Indices for entry on sample sheet		
Unique dual-index number	**i7 bases for entry on sample sheet (all instruments)**	**i5 bases for entry on sample sheet (MiSeq, HiSeq 2000/2500, NovaSeq 6000)**	**i5 bases for entry on sample sheet (iSeq 100, MiniSeq, NextSeq, HiSeq X, HiSeq 3000/4000)**
UDI 001	TGAACGTTGT	ATGGCCGACT	AGTCGGCCAT
UDI 002	ACCAGACTTG	CGATGAGCAC	GTGCTCATCG
UDI 003	ACTGGCGAAC	GATAAGTCGA	TCGACTTATC
UDI 004	GCGTTAGGCA	TCACGCCTTG	CAAGGCGTGA
UDI 005	TTATCGGCCT	AGGAACACAA	TTGTGTTCCT
UDI 006	GAGGTATAAG	CTCAGTAGGC	GCCTACTGAG
UDI 007	TCAAGGATTC	GAAGTGCCTG	CAGGCACTTC
UDI 008	CGAACCGAGA	TCTCTCGCCT	AGGCGAGAGA
UDI 009	GAGCCAAGTT	AGGCACCTTC	GAAGGTGCCT
UDI 010	AAGGCCGTAG	CTGTTGGTAA	TTACCAACAG
UDI 011	TTAGAGAAGC	GCTGGTACCT	AGGTACCAGC
UDI 012	TCTAAGACCA	TAAGGAGCGG	CCGCTCCTTA
UDI 013	TGTAACCACT	AATCGCTCCA	TGGAGCGATT
UDI 014	CCGACACAAG	CTCCTAATTG	CAATTAGGAG
UDI 015	CTCTGATGGC	GCCTCATAAT	ATTATGAGGC
UDI 016	CGGCCTGTTA	TGTATTGAGC	GCTCAATACA
UDI 017	TGCATAGCTT	AGCCATAACA	TGTTATGGCT
UDI 018	AACCTTCTCG	CCACAAGTGG	CCACTTGTGG
UDI 019	AAGAGATCAC	GTTATCACAC	GTGTGATAAC
UDI 020	GCCTGAAGGA	TACCGTTCTT	AAGAACGGTA

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Indices for entry on sample sheet			
Unique dual-index number	**i7 bases for entry on sample sheet (all instruments)**	**i5 bases for entry on sample sheet (MiSeq, HiSeq 2000/2500, NovaSeq 6000)**	**i5 bases for entry on sample sheet (iSeq 100, MiniSeq, NextSeq, HiSeq X, HiSeq 3000/4000)**
UDI 021	ATTGTGCCTT	AGGCGTTAGG	CCTAACGCCT
UDI 022	TCCTCTACCG	CCGTAACGTC	GACGTTACGG
UDI 023	TACCATGAAC	GTAATAGCCA	TGGCTATTAC
UDI 024	CATTGGCAGA	TAGCGCCGAT	ATCGGCGCTA
UDI 025	CACTGCTATT	CATTCTTGGA	TCCAAGAATG
UDI 026	AATGGTAGGT	ATGCAAGGTT	AACCTTGCAT
UDI 027	GATACCTATG	CGCCAGACAA	TTGTCTGGCG
UDI 028	CACTAGGTAC	GAAGGTTGGC	GCCAACCTTC
UDI 029	AGCTCGTTCA	TCGCATCACG	CGTGATGCGA
UDI 030	TGTCAGTCTT	CCGGTCATGA	TCATGACCGG
UDI 031	GATGAACAGT	ATTCACAAGC	GCTTGTGAAT
UDI 032	ACAATCGGCG	CAACCTGTAA	TTACAGGTTG
UDI 033	GATTGAGTTC	GCCAGTCGTT	AACGACTGGC
UDI 034	GTAATGCCAA	TGCCTTGTCG	CGACAAGGCA
UDI 035	TCGTTGCGCT	CTATCCGCTG	CAGCGGATAG
UDI 036	AGGTGAGTAT	AATGCCGGAA	TTCCGGCATT
UDI 037	TCGATAATGG	CGGTTATCCG	CGGATAACCG
UDI 038	GCGTCTCTTC	GCGGAAGAGT	ACTCTTCCGC
UDI 039	GTCTCCTGCA	TTGGTTAGTC	GACTAACCAA
UDI 040	GAGCTTCATT	TTCAGTGTGA	TCACACTGAA

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Indices for entry on sample sheet			
Unique dual-index number	**i7 bases for entry on sample sheet (all instruments)**	**i5 bases for entry on sample sheet (MiSeq, HiSeq 2000/2500, NovaSeq 6000)**	**i5 bases for entry on sample sheet (iSeq 100, MiniSeq, NextSeq, HiSeq X, HiSeq 3000/4000)**
UDI 041	AGGCCTACAT	AGAATTCTGG	CCAGAATTCT
UDI 042	TGTGGAACCG	CATTGACTCT	AGAGTCAATG
UDI 043	CGTATTAAGC	GCGGCTTCAA	TTGAAGCCGC
UDI 044	CCAGTGGTTA	TTATGGTCTC	GAGACCATAA
UDI 045	GCGTTCGAGT	CGTAACCAGG	CCTGGTTACG
UDI 046	CCTTCCGGTT	AGCTCAGATA	TATCTGAGCT
UDI 047	CACAAGACGG	CCGGTGTTAC	GTAACACCGG
UDI 048	GCTTACACAC	GACCTAACCT	AGGTTAGGTC
UDI 049	AGGATGTCCA	TTGTAGAAGG	CCTTCTACAA
UDI 050	CACCTTATGT	CCTAGCACTA	TAGTGCTAGG
UDI 051	AAGCGGCTGT	ATCGTGTTCT	AGAACACGAT
UDI 052	TTCCTGTGAG	CCAACTTATC	GATAAGTTGG
UDI 053	AGTACAGTTC	GAAGCCAAGG	CCTTGGCTTC
UDI 054	TACAGCCTCA	TGGAGTTCAA	TTGAACTCCA
UDI 055	GTTCTATTGG	CTTCAATCCT	AGGATTGAAG
UDI 056	ATATACCGGT	ATCTTGCGTG	CACGCAAGAT
UDI 057	CCTCGGAATG	CGTCTAAGGT	ACCTTAGACG
UDI 058	GTTCTGGAAC	GAGGTGAACA	TGTTCACCTC
UDI 059	AGATTCACCA	TCAGAACTAC	GTAGTTCTGA
UDI 060	TCGGTCAGAT	CGGATATTGA	TCAATATCCG

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Indices for entry on sample sheet			
Unique dual-index number	**i7 bases for entry on sample sheet (all instruments)**	**i5 bases for entry on sample sheet (MiSeq, HiSeq 2000/2500, NovaSeq 6000)**	**i5 bases for entry on sample sheet (iSeq 100, MiniSeq, NextSeq, HiSeq X, HiSeq 3000/4000)**
UDI 061	CACTCTCGCT	AGGAGTAGAT	ATCTACTCCT
UDI 062	GTTGGTCCAG	CCGCCGAATA	TATTCGGCGG
UDI 063	AGCTCGAAGC	GAGTCTATAC	GTATAGACTC
UDI 064	AGAGGTTCTA	TTATTACCGG	CCGGTAATAA
UDI 065	ATGACTCGAA	CGCTCGTTAG	CTAACGAGCG
UDI 066	GAACAATCCT	AACAACGCTG	CAGCGTTGTT
UDI 067	TGGCAAGGAG	CGCGGCTATT	AATAGCCGCG
UDI 068	GAATATTGGC	GCTCGACACA	TGTGTCGAGC
UDI 069	CCGGAACCTA	TTCTTCCAAC	GTTGGAAGAA
UDI 070	ACTTGTTCGG	TTGGCGGTTG	CAACCGCCAA
UDI 071	CAAGTCCAAT	AACAGGCAAT	ATTGCCTGTT
UDI 072	AACCGCAAGG	CAGAATGGCG	CGCCATTCTG
UDI 073	ACGTTGACTC	GTTGAGATTC	GAATCTCAAC
UDI 074	CCACTTAACA	TGTGTGCGGA	TCCGCACACA
UDI 075	AGCAGTTCCT	GTTCGGCGAA	TTCGCCGAAC
UDI 076	TCGCCTTCGT	AGCTGTATTG	CAATACAGCT
UDI 077	TAGGACTGCG	CAGCGGATGA	TCATCCGCTG
UDI 078	TCCGAGCGAA	GTCCTTGGAT	ATCCAAGGAC
UDI 079	TTCGGTTGTT	TCTAGATGCT	AGCATCTAGA
UDI 080	ACAGGAGGAA	CGAGCCACAT	ATGTGGCTCG

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Indices for entry on sample sheet			
Unique dual-index number	**i7 bases for entry on sample sheet (all instruments)**	**i5 bases for entry on sample sheet (MiSeq, HiSeq 2000/2500, NovaSeq 6000)**	**i5 bases for entry on sample sheet (iSeq 100, MiniSeq, NextSeq, HiSeq X, HiSeq 3000/4000)**
UDI 081	CCTCCATTAA	ATGGAATGGA	TCCATTCCAT
UDI 082	AGTCGCGGTT	CATTCCTCAC	GTGAGGAATG
UDI 083	CTCATCCAGG	GCATAGGAAG	CTTCCTATGC
UDI 084	TGTGGTTGAA	TGTTCGTGTT	AACACGAACA
UDI 085	TTATGCGTGG	TAAGACCGTT	AACGGTCTTA
UDI 086	GCGAATGTAT	ATGGTACCAG	CTGGTACCAT
UDI 087	GTCAAGCTCG	CCGACAGCTT	AAGCTGTCGG
UDI 088	TAGAGTTGGA	GACGATATGA	TCATATCGTC
UDI 089	CTGATGATCT	TTGTACTCCA	TGGAGTACAA
UDI 090	ACTAGGTGTT	GTGCACATAA	TTATGTGCAC
UDI 091	CTGTTAGCGG	AGGACAAGTA	TACTTGTCCT
UDI 092	ATCGCACCAA	CCGATTCGAG	CTCGAATCGG
UDI 093	CTTACTTGGT	GTAGGAACTT	AAGTTCCTAC
UDI 094	CCTTAATGCG	TACACTACGA	TCGTAGTGTA
UDI 095	TCTCGCCTAG	ATGACCTTGA	TCAAGGTCAT
UDI 096	TCTTCAGAGA	CTACGTGACG	CGTCACGTAG
UDI 097	TACCGGTGGT	AACAATCAGG	CCTGATTGTT
UDI 098	AGGTGTTACG	CTGGTGTGCA	TGCACACCAG
UDI 099	ACAGACCGAC	GCATATCCTT	AAGGATATGC
UDI 100	CGAATACGTA	TGTCCTGTAC	GTACAGGACA

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Indices for entry on sample sheet			
Unique dual-index number	**i7 bases for entry on sample sheet (all instruments)**	**i5 bases for entry on sample sheet (MiSeq, HiSeq 2000/2500, NovaSeq 6000)**	**i5 bases for entry on sample sheet (iSeq 100, MiniSeq, NextSeq, HiSeq X, HiSeq 3000/4000)**
UDI 101	TAGCATCGAT	AGAACGTCGC	GCGACGTTCT
UDI 102	CCATGAGTCG	CACGGACTAG	CTAGTCCGTG
UDI 103	ACTAACATGC	GTTGAACACT	AGTGTTCAAC
UDI 104	ACACTCTCTA	TCGCGTGGTA	TACCACGCGA
UDI 105	GCTCTTGCCT	AGCCACTATG	CATAGTGGCT
UDI 106	AATCTTGAGG	CCACCTACCA	TGGTAGGTGG
UDI 107	CTTAACGGTC	GTTCCGGTGT	ACACCGGAAC
UDI 108	TTGTGACCAA	TAGGTCTGAC	GTCAGACCTA
UDI 109	TCACACACCT	AGGAAGCATT	AATGCTTCCT
UDI 110	CTGCAATTAG	CCTTAGTTGG	CCAACTAAGG
UDI 111	CTCCTTACTC	GTCCTATTCA	TGAATAGGAC
UDI 112	GCAACGCAGA	TAAGATGGAC	GTCCATCTTA
UDI 113	CCTTACCAAT	AGGCCATGGT	ACCATGGCCT
UDI 114	TTAATCCTCG	CATTGGCCAA	TTGGCCAATG
UDI 115	TTCCGAGTTC	GCTATGAATC	GATTCATAGC
UDI 116	CTCGAGAGGA	TTGGTCCTCG	CGAGGACCAA
UDI 117	TGTTGGCTGT	AGCGACATAC	GTATGTCGCT
UDI 118	CGTATCTGCG	CAAGTAGTCT	AGACTACTTG
UDI 119	CCATAGTATC	GTCAAGAAGA	TCTTCTTGAC
UDI 120	TGGACAGTAA	TCCTGTTATG	CATAACAGGA

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Indices for entry on sample sheet			
Unique dual-index number	**i7 bases for entry on sample sheet (all instruments)**	**i5 bases for entry on sample sheet (MiSeq, HiSeq 2000/2500, NovaSeq 6000)**	**i5 bases for entry on sample sheet (iSeq 100, MiniSeq, NextSeq, HiSeq X, HiSeq 3000/4000)**
UDI 121	GTACCTTGTT	AAGTGCGATA	TATCGCACTT
UDI 122	GAGTGCCTCT	AGGCTACACG	CGTGTAGCCT
UDI 123	TAAGTAGCGG	CTATATCGGC	GCCGATATAG
UDI 124	CGTGGTGTTC	GCTAAGGTAA	TTACCTTAGC
UDI 125	CATTCCTGAA	TAACCTGGTT	AACCAGGTTA
UDI 126	AAGATGCATG	AGTTGGTCTA	TAGACCAACT
UDI 127	CCTTGGAGCT	ATGCAGCTGG	CCAGCTGCAT
UDI 128	ACCGGAACAG	CGTTGCCTTC	GAAGGCAACG
UDI 129	GAATGGAAGC	GCGTGGAGAA	TTCTCCACGC
UDI 130	GTTCTCCATA	TACGCCTCCT	AGGAGGCGTA
UDI 131	GTCACTATGT	AATTCGGTAG	CTACCGAATT
UDI 132	TGGTAGAACT	ATTGTCGAAC	GTTCGACAAT
UDI 133	ACGCCTATGG	CAACCTTGCG	CGCAAGGTTG
UDI 134	AATCCGTTAC	GCACTGCGTA	TACGCAGTGC
UDI 135	GTTGAGGCTA	TGCTAGTAGT	ACTACTAGCA
UDI 136	TATCAACTGG	AAGTCACGGA	TCCGTGACTT
UDI 137	AAGAGGAGAT	AGCGATTGAA	TTCAATCGCT
UDI 138	GTCTTCTCGG	CTACCTCTCT	AGAGAGGTAG
UDI 139	GAAGCCACTC	GACAACTGTC	GACAGTTGTC
UDI 140	GTAGGACACA	TCCATTGCGG	CCGCAATGGA

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Indices for entry on sample sheet			
Unique dual-index number	**i7 bases for entry on sample sheet (all instruments)**	**i5 bases for entry on sample sheet (MiSeq, HiSeq 2000/2500, NovaSeq 6000)**	**i5 bases for entry on sample sheet (iSeq 100, MiniSeq, NextSeq, HiSeq X, HiSeq 3000/4000)**
UDI 141	CTCCTCGTAT	AGCCTCGCAA	TTGCGAGGCT
UDI 142	CCACATGATT	AATACAGGCT	AGCCTGTATT
UDI 143	AGACGGTTGG	CGGACCGTTA	TAACGGTCCG
UDI 144	CTAGGTTGAC	GCGCTTATGC	GCATAAGCGC
UDI 145	AAGCGTACCA	TTAACACGAG	CTCGTGTTAA
UDI 146	TCATGTTGGT	CGCCTCTAGA	TCTAGAGGCG
UDI 147	TTGGAATGGT	AATCGACCTT	AAGGTCGATT
UDI 148	GTGTATGTTG	CCGCAATAAC	GTTATTGCGG
UDI 149	TCCTGTCAAC	GTTCCAACGA	TCGTTGGAAC
UDI 150	TAATCAGGCA	TGTTAGACCG	CGGTCTAACA
UDI 151	GTAGTGGATT	AACCTCATAG	CTATGAGGTT
UDI 152	AATTGCGCAT	ATGAATCCAC	GTGGATTCAT
UDI 153	GACAATAACG	CGGCTTAATT	AATTAAGCCG
UDI 154	ACAGTTAAGC	GAGTTGCAGG	CCTGCAACTC
UDI 155	AGCCACACTA	TCCACGAACA	TGTTCGTGGA
UDI 156	CAATCGTCTT	TGACGGAGGA	TCCTCCGTCA
UDI 157	AGGAGCTTGT	AATGAGTACG	CGTACTCATT
UDI 158	TTGAGCGGAG	CGTCTTCCGA	TCGGAAGACG
UDI 159	AGTAGCTCTC	GACAGAGATT	AATCTCTGTC
UDI 160	CACGCTGTCA	TTACGCTAAC	GTTAGCGTAA

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Indices for entry on sample sheet			
Unique dual-index number	**i7 bases for entry on sample sheet (all instruments)**	**i5 bases for entry on sample sheet (MiSeq, HiSeq 2000/2500, NovaSeq 6000)**	**i5 bases for entry on sample sheet (iSeq 100, MiniSeq, NextSeq, HiSeq X, HiSeq 3000/4000)**
UDI 161	AAGACCTCTT	CTCCTCGAAG	CTTCGAGGAG
UDI 162	GACCTCTTCT	ATACCGCAGA	TCTGCGGTAT
UDI 163	TACTTCCTTG	CCTATCTGAT	ATCAGATAGG
UDI 164	TGCGATACGC	GATCGGTTAC	GTAACCGATC
UDI 165	GCAGGCTTAA	TGGTGAGGTG	CACCTCACCA
UDI 166	TAAGCTTGTG	AACCGGCGTA	TACGCCGGTT
UDI 167	ATGGTCCGCT	AATACCGATC	GATCGGTATT
UDI 168	ATGTCAGAAG	CGATACTCAA	TTGAGTATCG
UDI 169	GACGAAGGTC	GTAAGGCGGT	ACCGCCTTAC
UDI 170	ATCACCGTGA	TTCAAGGTCG	CGACCTTGAA
UDI 171	GCTACAGTGT	TATCCGAGTA	TACTCGGATA
UDI 172	CGTCGAATAT	AGCGCGCTTA	TAAGCGCGCT
UDI 173	CAACCATCGG	CCGGAGACAT	ATGTCTCCGG
UDI 174	CGGTCCATTC	GAGATAACTG	CAGTTATCTC
UDI 175	AGAAGAGCCA	TTGTAAGCGC	GCGCTTACAA
UDI 176	CTATGCAATG	CAAGAGGAGG	CCTCCTCTTG
UDI 177	CACTGAACCG	AACCTTAGGA	TCCTAAGGTT
UDI 178	TACTGTGTGA	CTGGCAACTC	GAGTTGCCAG
UDI 179	GCATTCTGTT	GAACTTGTTG	CAACAAGTTC
UDI 180	CTCCGCTAAG	TGTGCAAGAT	ATCTTGCACA

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Indices for entry on sample sheet			
Unique dual-index number	**i7 bases for entry on sample sheet (all instruments)**	**i5 bases for entry on sample sheet (MiSeq, HiSeq 2000/2500, NovaSeq 6000)**	**i5 bases for entry on sample sheet (iSeq 100, MiniSeq, NextSeq, HiSeq X, HiSeq 3000/4000)**
UDI 181	TCGCTTGAGA	AATCGAGAGA	TCTCTCGATT
UDI 182	AACTAGCCTT	AGCGTGTCAG	CTGACACGCT
UDI 183	TTCGCTCAGG	CTTGGTGATT	AATCACCAAG
UDI 184	CTCTACAACA	GAAGCAGCAA	TTGCTGCTTC
UDI 185	TGAGTGTGTT	TTCCGTCGAC	GTCGACGGAA
UDI 186	TAGTTAGTCG	CGAGATGCCA	TGGCATCTCG
UDI 187	GCCTGATCCT	AAGTTCGTGC	GCACGAACTT
UDI 188	CGAGTACAGG	CGTCCATAAG	CTTATGGACG
UDI 189	GCCTAGATTA	TTGTGGCATA	TATGCCACAA
UDI 190	TCGGCACTGT	AGATCGGAAT	ATTCCGATCT
UDI 191	CCGTGCAAGA	CATTCTACTG	CAGTAGAATG
UDI 192	CTGGCTGGTT	ATCGCCGTAG	CTACGGCGAT
UDI 193	CGTTAGGATT	ATCCTTACAC	GTGTAAGGAT
UDI 194	TTCCATTACG	CGCAAGGACT	AGTCCTTGCG
UDI 195	TAGCGGTAAC	GCTGGCGTTA	TAACGCCAGC
UDI 196	GTAGCCAGGA	TACTTAGAGG	CCTCTAAGTA
UDI 197	AGGATACTCT	ATGGCGATGC	GCATCGCCAT
UDI 198	TATCCTCCAG	CATTGGTGCG	CGCACCAATG
UDI 199	TAAGTCGTTC	GCGAGATATA	TATATCTCGC
UDI 200	TCCGGATTGA	TGACTGCTAT	ATAGCAGTCA

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Indices for entry on sample sheet			
Unique dual-index number	**i7 bases for entry on sample sheet (all instruments)**	**i5 bases for entry on sample sheet (MiSeq, HiSeq 2000/2500, NovaSeq 6000)**	**i5 bases for entry on sample sheet (iSeq 100, MiniSeq, NextSeq, HiSeq X, HiSeq 3000/4000)**
UDI 201	ACGTCTTGTT	AACGTCCGCT	AGCGGACGTT
UDI 202	ATGAAGTGCG	CGCACATGTC	GACATGTGCG
UDI 203	CGATCACTGC	GCACACCTGA	TCAGGTGTGC
UDI 204	CCTATCGGAA	TTGTCCAGAG	CTCTGGACAA
UDI 205	CAGAGAGCTT	AGCCTTCCTG	CAGGAAGGCT
UDI 206	GCAACTTGCG	CCTTACGCCA	TGGCGTAAGG
UDI 207	TATGGAGGAC	GAATACGTAC	GTACGTATTC
UDI 208	TGAGATCAGA	TTGGCACCGT	ACGGTGCCAA
UDI 209	TCAGCCTATT	ATTAGGTGGC	GCCACCTAAT
UDI 210	GTTGTGAGCG	CGATCAAGAA	TTCTTGATCG
UDI 211	TCAGTAACAC	GCTGTCTTCT	AGAAGACAGC
UDI 212	AAGGCTCAGA	TACATGTCTG	CAGACATGTA
UDI 213	GTGTGGTGGT	AACCAGTTGA	TCAACTGGTT
UDI 214	CCGAGCTTAG	CCGGTAAGCT	AGCTTACCGG
UDI 215	ATCACGCTTC	GTTCGAATAG	CTATTCGAAC
UDI 216	TAGCTATGCA	TGTCAGGCTC	GAGCCTGACA
UDI 217	TGTTCCTCAT	CAACAGTGTT	AACACTGTTG
UDI 218	CATACCTTCT	AAGAGAGGAA	TTCCTCTCTT
UDI 219	GCCTTCAATG	CGGTTGTAGC	GCTACAACCG
UDI 220	CTTGACCAGC	GCCTGAAGTG	CACTTCAGGC

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Indices for entry on sample sheet			
Unique dual-index number	**i7 bases for entry on sample sheet (all instruments)**	**i5 bases for entry on sample sheet (MiSeq, HiSeq 2000/2500, NovaSeq 6000)**	**i5 bases for entry on sample sheet (iSeq 100, MiniSeq, NextSeq, HiSeq X, HiSeq 3000/4000)**
UDI 221	CTACACACAA	TTACGACACT	AGTGTCGTAA
UDI 222	TAGGCTGAAT	CGCCTAGATC	GATCTAGGCG
UDI 223	TCGGAGTCCT	AATCTGGATG	CATCCAGATT
UDI 224	AACATCGCGG	CGACGGTACA	TGTACCGTCG
UDI 225	GTTGTCTTAC	GTAGTATTGC	GCAATACTAC
UDI 226	GTGGCAACTA	TCCAGCGGAT	ATCCGCTGGA
UDI 227	GAGCAGGCAT	CAACCACCTC	GAGGTGGTTG
UDI 228	AACGGCACCT	AGCTTAGGCG	CGCCTAAGCT
UDI 229	AGTAACCTTG	CCGGTTCCTT	AAGGAACCGG
UDI 230	TCTCATAAGC	GACATTGAAC	GTTCAATGTC
UDI 231	TGCTTGCCAA	TTAGAGGCGA	TCGCCTCTAA
UDI 232	CGGTTCCTGT	CAAGCCGAAC	GTTCGGCTTG
UDI 233	CCAAGTAGAT	AGGAGAACGG	CCGTTCTCCT
UDI 234	AAGGTTGGCG	CCTGTTAGAC	GTCTAACAGG
UDI 235	TGCTCTGGTC	GTTCTACGTT	AACGTAGAAC
UDI 236	ACTGTAACGA	TAAGTCCACA	TGTGGACTTA
UDI 237	GATTCCAGGT	CAAGAACCAT	ATGGTTCTTG
UDI 238	TTCACCAGAT	AGTTGATGAC	GTCATCAACT
UDI 239	ACTTCCAAGG	CCTACTCTTG	CAAGAGTAGG
UDI 240	CCGAATATTC	GAACAATCCA	TGGATTGTTC

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Indices for entry on sample sheet			
Unique dual-index number	**i7 bases for entry on sample sheet (all instruments)**	**i5 bases for entry on sample sheet (MiSeq, HiSeq 2000/2500, NovaSeq 6000)**	**i5 bases for entry on sample sheet (iSeq 100, MiniSeq, NextSeq, HiSeq X, HiSeq 3000/4000)**
UDI 241	CTCTTATCCA	TTCTGTTGGT	ACCAACAGAA
UDI 242	TCACAGCGGT	CATCGTCAGG	CCTGACGATG
UDI 243	CCTCTGTCGT	ATGCATGAAG	CTTCATGCAT
UDI 244	TCTGTTCTCG	CGTGAATCGC	GCGATTCACG
UDI 245	GATACTTCAC	GAGCAGCCTT	AAGGCTGCTC
UDI 246	AGTGCTGATA	TCGATTACCA	TGGTAATCGA
UDI 247	ATCCTTCGGT	CAGTCCAATT	AATTGGACTG
UDI 248	GACAACGATT	AGAGGCTTGG	CCAAGCCTCT
UDI 249	GAACCGGTAG	CAGGCTCTCA	TGAGAGCCTG
UDI 250	AGCAATGAGC	GTTCGCTCTC	GAGAGCGAAC
UDI 251	CAAGACTCCA	TCGGACTAAT	ATTAGTCCGA
UDI 252	ACCGTGTAGG	CGAGATCTTC	GAAGATCTCG
UDI 253	AGGCACAGGT	ATAACCGGAC	GTCCGGTTAT
UDI 254	CGACAGATCG	CGTGTAGTTA	TAACTACACG
UDI 255	ACGCGACAAC	GAACATAGGT	ACCTATGTTC
UDI 256	ACTTGCGTTA	TCTAACATCG	CGATGTTAGA
UDI 257	CACCACTCAT	AACGGTGGCA	TGCCACCGTT
UDI 258	CTTCGTAACT	AGGACGGTGT	ACACCGTCCT
UDI 259	CAGTATTCGG	CTGTGACCTG	CAGGTCACAG
UDI 260	CAGTCTGGAC	GCTGTAACAA	TTGTTACAGC

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Indices for entry on sample sheet			
Unique dual-index number	**i7 bases for entry on sample sheet (all instruments)**	**i5 bases for entry on sample sheet (MiSeq, HiSeq 2000/2500, NovaSeq 6000)**	**i5 bases for entry on sample sheet (iSeq 100, MiniSeq, NextSeq, HiSeq X, HiSeq 3000/4000)**
UDI 261	TACCGTTCTA	TACGGACGTC	GACGTCCGTA
UDI 262	GTGTCCACAG	CCTAAGGAGC	GCTCCTTAGG
UDI 263	TTACGACTGT	ATAAGGCCAG	CTGGCCTTAT
UDI 264	GACGCGAATG	CTCATCTGTA	TACAGATGAG
UDI 265	CAACGTACGC	GAAGGCATCT	AGATGCCTTC
UDI 266	AGCTCAGGAA	TCTCTACTGC	GCAGTAGAGA
UDI 267	GATAGGCGGT	AACCGAACAA	TTGTTCGGTT
UDI 268	AGTAGGAAGT	ATCTCGCCAC	GTGGCGAGAT
UDI 269	CATGTTGTAG	CCATGCAACG	CGTTGCATGG
UDI 270	CACATTCTTC	GAATGGTGTA	TACACCATTC
UDI 271	GCAGCTCGTA	TATATGCCGT	ACGGCATATA
UDI 272	GTTCAGACGG	CTCGATAGAT	ATCTATCGAG
UDI 273	TCCTGGAAGT	AACACAAGAG	CTCTTGTGTT
UDI 274	GCATTGTTAG	CGCAATCGGT	ACCGATTGCG
UDI 275	GACCTACAGC	GTTGCGTAGA	TCTACGCAAC
UDI 276	CACCGACGTA	TAGAGTGATC	GATCACTCTA
UDI 277	CTCTCACCTT	AAGACGCAGC	GCTGCGTCTT
UDI 278	CTCGTTCATT	AACTTCTCGA	TCGAGAAGTT
UDI 279	TGGTGGCAAG	CGCAACTGAG	CTCAGTTGCG
UDI 280	GATTGCTTGA	GCTCCGCAAT	ATTGCGGAGC

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Unique dual-index number	Indices for entry on sample sheet: i7 bases for entry on sample sheet (all instruments)	i5 bases for entry on sample sheet (MiSeq, HiSeq 2000/2500, NovaSeq 6000)	i5 bases for entry on sample sheet (iSeq 100, MiniSeq, NextSeq, HiSeq X, HiSeq 3000/4000)
UDI 281	CCGTTAAGGT	GTAACTTCCG	CGGAAGTTAC
UDI 282	TGCTGAGAGG	CTCACGACTA	TAGTCGTGAG
UDI 283	TTGTCACTTG	AACCAACGGC	GCCGTTGGTT
UDI 284	GCTGTTATGT	CCTGCCTGTA	TACAGGCAGG
UDI 285	GCAGCAGTTG	TACGCTGCAG	CTGCAGCGTA
UDI 286	GCAGATCAAT	AATGTTGCGA	TCGCAACATT
UDI 287	TGGTTCACGG	CGACGTTCTG	CAGAACGTCG
UDI 288	TCGACCGCAT	AATAGGACAC	GTGTCCTATT
UDI 289	TAACCTAGGT	ATGTGCCTCA	TGAGGCACAT
UDI 290	AACTCATGCG	CGACTCCGTT	AACGGAGTCG
UDI 291	CCGGATGAAC	GCTGTTGTGG	CCACAACAGC
UDI 292	CGTTGCCGTA	TACCAATCAC	GTGATTGGTA
UDI 293	GCTCTACGGT	ATGTCTTACG	CGTAAGACAT
UDI 294	TGCATTGGCG	CGCAACAATA	TATTGTTGCG
UDI 295	CGATTGTGAC	GAACGAAGAC	GTCTTCGTTC
UDI 296	GACTGCACTA	TCGAGGACGT	ACGTCCTCGA
UDI 297	GTTAACTGCT	ATTATGAGCG	CGCTCATAAT
UDI 298	TCGGACCTTG	CGCGTTATAA	TTATAACGCG
UDI 299	TGCAGCAAGC	GCGTGCATGT	ACATGCACGC
UDI 300	CACATGCGAA	TAAGCGGCTC	GAGCCGCTTA

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Indices for entry on sample sheet			
Unique dual-index number	**i7 bases for entry on sample sheet (all instruments)**	**i5 bases for entry on sample sheet (MiSeq, HiSeq 2000/2500, NovaSeq 6000)**	**i5 bases for entry on sample sheet (iSeq 100, MiniSeq, NextSeq, HiSeq X, HiSeq 3000/4000)**
UDI 301	CAGACGTAAT	AACATGGAGA	TCTCCATGTT
UDI 302	ATTCGGTACG	CCGAGTCTCT	AGAGACTCGG
UDI 303	TTAGCACGGC	GTACTTCTAC	GTAGAAGTAC
UDI 304	GAGGATAGTA	TGTTCACATG	CATGTGAACA
UDI 305	AACTGTGGTT	AAGGTAACGC	GCGTTACCTT
UDI 306	ATTACCTCGG	CCGCCTTACT	AGTAAGGCGG
UDI 307	CGCTGTATAC	GTTGAGGCAG	CTGCCTCAAC
UDI 308	CTTGCTCACA	TGGCGACCTA	TAGGTCGCCA
UDI 309	CAACACCTGT	AGAAGCGACA	TGTCGCTTCT
UDI 310	CAATTGCTCG	CAGGATAATC	GATTATCCTG
UDI 311	CATAGACAAC	GCTCCTACAG	CTGTAGGAGC
UDI 312	TTGGTGTCTA	TTCAACAGGT	ACCTGTTGAA
UDI 313	TATGTCCTGT	CCTCGTCCAT	ATGGACGAGG
UDI 314	GCCAATTCGT	AGCGTTGGTT	AACCAACGCT
UDI 315	TAGGCGATCG	CATTCGAACA	TGTTCGAATG
UDI 316	ATGAGTGTAC	GCTTACCGAC	GTCGGTAAGC
UDI 317	CCGAAGGATA	TTAGCTTAGG	CCTAAGCTAA
UDI 318	AGTCCACTGT	CCGACACACA	TGTGTGTCGG
UDI 319	GCGGCTAATT	ATTCGCTGAT	ATCAGCGAAT
UDI 320	TCTAACTCAG	CCAAGAGGCA	TGCCTCTTGG

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Indices for entry on sample sheet			
Unique dual-index number	**i7 bases for entry on sample sheet (all instruments)**	**i5 bases for entry on sample sheet (MiSeq, HiSeq 2000/2500, NovaSeq 6000)**	**i5 bases for entry on sample sheet (iSeq 100, MiniSeq, NextSeq, HiSeq X, HiSeq 3000/4000)**
UDI 321	CAAGCTGAGC	GACGCAGTTC	GAACTGCGTC
UDI 322	CCAGAGCACA	TGGAACTCGG	CCGAGTTCCA
UDI 323	TGTACAAGGT	CCACACCAAT	ATTGGTGTGG
UDI 324	TAGAATGCCT	AGTTCTCGGC	GCCGAGAACT
UDI 325	TGTCTTACTG	CTTGACGACG	CGTCGTCAAG
UDI 326	ATGACTAAGC	GAGGTCGCTA	TAGCGACCTC
UDI 327	ATGTAGGCAA	TCAGTAGCAT	ATGCTACTGA
UDI 328	GCGAAGAGGT	CTAACGTGGA	TCCACGTTAG
UDI 329	CGGTGGTTCT	ATGCCAACCG	CGGTTGGCAT
UDI 330	CTGTCGTTGG	CGGTCGATTC	GAATCGACCG
UDI 331	TGATCGACAC	GAAGTACAGT	ACTGTACTTC
UDI 332	CCACCAGCTA	TCTGCAGTAA	TTACTGCAGA
UDI 333	CACGGTTCGT	CTATCCTAGC	GCTAGGATAG
UDI 334	AGTGAGAGCT	AACACTCCTT	AAGGAGTGTT
UDI 335	TTGCATGCGG	CCGAACCTAA	TTAGGTTCGG
UDI 336	TATACGTGTC	GTCTAGTCGC	GCGACTAGAC
UDI 337	TGACGCGTTA	TGGATGTACG	CGTACATCCA
UDI 338	TACAGAACGT	CTACCAGCGT	ACGCTGGTAG
UDI 339	CTTGTCAGGT	AAGGATTCAG	CTGAATCCTT
UDI 340	ATCCACAGCG	CGAGGTGTGT	ACACACCTCG

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Indices for entry on sample sheet			
Unique dual-index number	**i7 bases for entry on sample sheet (all instruments)**	**i5 bases for entry on sample sheet (MiSeq, HiSeq 2000/2500, NovaSeq 6000)**	**i5 bases for entry on sample sheet (iSeq 100, MiniSeq, NextSeq, HiSeq X, HiSeq 3000/4000)**
UDI 341	CCTATCCATC	GTAGACGCTC	GAGCGTCTAC
UDI 342	ACCGCGAGTA	TCGTCCGTCA	TGACGGACGA
UDI 343	AAGTTCTGGT	CCGTGATAGG	CCTATCACGG
UDI 344	ACAGGTATCG	AGGATGACCT	AGGTCATCCT
UDI 345	ATGACGGATT	CCTCGAGTAC	GTACTCGAGG
UDI 346	GTCTGAGTAG	GTCACTGAGG	CCTCAGTGAC
UDI 347	TGCCAGATGT	TACGGTTAGA	TCTAACCGTA
UDI 348	GCTAAGCATT	CAACGAGAAT	ATTCTCGTTG
UDI 349	ACAGCATGGT	AATACACCGG	CCGGTGTATT
UDI 350	ATAGAGACCG	CCGATCCATC	GATGGATCGG
UDI 351	ATATCGCGTA	GAATCTCGCT	AGCGAGATTC
UDI 352	TTAAGGAGGT	TGACCGGCAA	TTGCCGGTCA
UDI 353	CTGTGCGACT	CATGATAGCA	TGCTATCATG
UDI 354	TCCGTATGCT	AACAGCTTCG	CGAAGCTGTT
UDI 355	CCATCGATGT	CTAGTGCTTA	TAAGCACTAG
UDI 356	GTGAGCCGTT	TGTGATACGT	ACGTATCACA
UDI 357	TGCCGTTAAT	ATGAGCGTAT	ATACGCTCAT
UDI 358	CGGATGTGGT	CTAGATATGG	CCATATCTAG
UDI 359	TCGCGTGTTG	CGCTATGCTG	CAGCATAGCG
UDI 360	CCGCGATCAT	TACTACGTGA	TCACGTAGTA

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Indices for entry on sample sheet			
Unique dual-index number	**i7 bases for entry on sample sheet (all instruments)**	**i5 bases for entry on sample sheet (MiSeq, HiSeq 2000/2500, NovaSeq 6000)**	**i5 bases for entry on sample sheet (iSeq 100, MiniSeq, NextSeq, HiSeq X, HiSeq 3000/4000)**
UDI 361	CGCGTTATCG	ATGTGGAGGT	ACCTCCACAT
UDI 362	GTAGCCTCCT	CCATGGCTCA	TGAGCCATGG
UDI 363	ACTAGACACT	CCAATCACGC	GCGTGATTGG
UDI 364	CGATTCGTTG	TTAGATCCAG	CTGGATCTAA
UDI 365	GAAGAGATGT	AGGAATATCG	CGATATTCCT
UDI 366	AGATCCGACG	CCTCCTATGT	ACATAGGAGG
UDI 367	CCAGGACATT	TAGAGACACG	CGTGTCTCTA
UDI 368	ACGTGGCATT	CCAGCTCAGT	ACTGAGCTGG
UDI 369	AAGCAGGACG	ATGGCTCATA	TATGAGCCAT
UDI 370	ACGAGTCGGT	CGGAGTGAAG	CTTCACTCCG
UDI 371	AGTGTACGCG	TACCTATGGT	ACCATAGGTA
UDI 372	ACCGACCATT	ATGAGACAGT	ACTGTCTCAT
UDI 373	TTGCTAACGT	CTAAGAGTTG	CAACTCTTAG
UDI 374	CTTGATACTG	TAACCGTATG	CATACGGTTA
UDI 375	CTGGATAAGT	AGAGTCCATG	CATGGACTCT
UDI 376	ATAGCTTACG	CTAGACCGCA	TGCGGTCTAG
UDI 377	GTCCATGAGT	TATGGCTTGT	ACAAGCCATA
UDI 378	ACTCCAGTCG	CGTTGTTCCT	AGGAACAACG
UDI 379	TCTCAGCACG	CCGACATTAG	CTAATGTCGG
UDI 380	ATCGTGATGT	TGTGAAGGCA	TGCCTTCACA

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Indices for entry on sample sheet			
Unique dual-index number	**i7 bases for entry on sample sheet (all instruments)**	**i5 bases for entry on sample sheet (MiSeq, HiSeq 2000/2500, NovaSeq 6000)**	**i5 bases for entry on sample sheet (iSeq 100, MiniSeq, NextSeq, HiSeq X, HiSeq 3000/4000)**
UDI 381	ACGCAATCCG	AGCATCGTCT	AGACGATGCT
UDI 382	GAGATCGGCT	CCGACTAGGA	TCCTAGTCGG
UDI 383	CTACGTCTCG	AACATTACCG	CGGTAATGTT
UDI 384	CTCAGGCTGT	CCTAATTCGT	ACGAATTAGG

Ordering Information

Product	Contents	Cat. no.
QIAseq xHYB (24)	For targeted enrichment from 24 microbial DNA samples. Kit contains reagents for first strand synthesis, second strand synthesis, ligation, library amplification, and hybrid capture.	333322 333332 333342 333352
QIAseq xHYB (96)	For targeted enrichment from 96 microbial DNA samples. Kit contains reagents for first strand synthesis, second strand synthesis, ligation, library amplification, and hybrid capture.	333325 333335 333345 333355
Related products		
QIAseq FX DNA Library UDI Kit (24)	Buffers and reagents for DNA fragmentation (including end repair and A-addition), ligation and library amplification; for use with Illumina instruments; includes a plate containing 24 Unique Dual Index Y-adapters.	180477
QIAseq FX DNA Library UDI Kit (96)	Buffers and reagents for DNA fragmentation (including end repair and A-addition), ligation, and library amplification; for use with Illumina instruments; includes a plate containing 96 Unique Dual Index Y-adapters.	180479 180480 180481 180482

Product	Contents	Cat. no.
QIAseq Library Quant Array Kit	Reagents for quantification of libraries prepared for Illumina or Ion Torrent® platforms; array format	333304
QIAseq Library Quant Assay Kit	Reagents for quantification of libraries prepared for Illumina or Ion Torrent platforms; assay format	333314
DNeasy PowerSoil Pro Kit (50)	For the isolation of microbial genomic DNA from all soil types	47014
QIAamp PowerFecal Pro DNA Kit (50)	For the isolation of DNA from stool, gut material, and biosolids	51804
QIAamp DNA Microbiome Kit (50)	For isolation of bacterial microbiome DNA from mixed samples	51704

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Document Revision History

Date	Changes
12/2021	Initial revision
02/2023	Added QIAseq Beads as a tool for drying down pooled libraries. Added a recommended starting point for wastewater samples in Pooling guidelines under Sequencing Setup on Illumina Instrument protocol. Updated an important point before starting under Appendix B.

Notes

Notes

Limited License Agreement for QIAseq xHYB Viral and Bacterial Library Kit

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